Thesis for doctoral degree (Ph.D.) 2010

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From THE DIVISION OF PERIODONTOLOGY,
DEPARTMENT OF DENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

THE EFFECT OF THERAPEUTIC AND ND:YAG LASER AS AN ADJUNCT TREATMENT MODALITY IN PERIODONTAL THERAPY

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200 Years
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Dedication

To my mother.
She died on the day that I received my DDS degree. She struggled with her disease until she knew that her son had finished his education and became a dentist.

Abstract

Laser irradiation has been proposed as an adjunct to conventional scaling and root planing in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

The present thesis is based on a series of four clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. Two different types of dental laser were investigated. Therapeutic lasers, which are claimed to stimulate cell regeneration and boost the immune system, were investigated in studies I and II: the general effect was investigated in Study I, while Study II compared the difference between gas and diode lasers in the same spectrum, in order to evaluate the importance of the length of coherence in biostimulation. In studies III and IV, the surgical Nd:YAG laser, which is usually applied for sulcular debridement and pocket decontamination, was evaluated in a novel approach. The test procedure comprised one single application of the laser with water coolant after conventional scaling and root planing. In study III, the outcome was evaluated after 3 months and in Study IV the long term outcome was evaluated, at least one year post-treatment.

The split mouth design was used in all four studies. Study I showed a better clinical outcome on the laser treated side and some improvement in immunological parameters. The results of Study II support the hypothesis that a laser with a long length of coherence is superior to one of a shorter length, although both lasers had some positive clinical effect. In Study III a single application of the Nd:YAG laser as an adjunct to scaling and root planing improved the short-term outcome and Study IV confirmed that this improvement was sustained.

In conclusion, the results of these studies confirm the potential role of laser irradiation as a non-invasive adjunctive to scaling and root planing in the treatment of periodontitis.

Key words: Low level laser, Nd:YAG laser, protease activity, coherence length, periodontal inflammation, cytokines, scaling and root planing.

LIST OF PUBLICATIONS

I. Qadri T, Miranda L, Tunér J, Gustafsson A. The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. J Clin Periodontol. 2005;32:714-719.

II. Qadri T, Bohdanecka P, Tunér J, Miranda L, Altamash M, Gustafsson A. The importance of coherence length in laser phototherapy of gingival inflammation: a pilot study. Lasers Med Sci. 2007;22:245-251.

III Qadri T, Poddani P, Javed F, Tunér J, Gustafsson A. A short-term evaluation of Nd:YAG laser as an adjunct to scaling and root planing in treatment of periodontal inflammation. J Periodontol. 2010;81:1161-1166.

IV Qadri T, Javed F, Poddani P, Tunér J, Gustafsson A. Long-term effects of a single application of a water-cooled pulsed Nd:YAG laser in supplement to scaling and root planing in patients with periodontal inflammation. Lasers Med Sci. 2010 Jun 27. [Epub ahead of print]

CONTENTS

LIST OF ABBREVIATIONS

Aa	Aggregatibacter actinomycetemcomitans
bFGF	Basic Fibroblast Growth Factor
cfu	Colony forming units
EMD	Enamel Matrix Protein derivate
Er:YAG	Erbium Yttrium Aluminium Garnet
GaAs	Gallium Arsenide
GCF	Gingival Crevicular Fluid
HeNe	Helium Neon
HSA	Human serum albumin
InGaAlP	Indium Gallium Aluminium Phosphide
LLLT	Low Level Laser Therapy
LPT	Laser phototherapy
mJ	Millijoule
mAbs	Milliabsorbance
MMP	Matrix metalloproteinase
Nd:YAG	Neodymium Yttrium Aluminium Garnet
ng	Nanogram
nm	Nanometer
ns	Nanoseconds
OPG	Osteoprotegerin
PBS	Phosphate buffered saline
pg	Porphyromonas gingivalis
PG	Prostaglandin
pg	Picogram
PMNL	Polymorphonuclear leukocytes
TGF	Transforming Growth factor

INTRODUCTION

LASER LIGHT

The word LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. The first such device, a ruby laser, was introduced by Maiman in 1960 (http://laserstars.org/history/ruby.html). According to the European Standard IEC 601, the definition of a laser is: ñAny device which can be made to produce or amplify electromagnetic radiation in the wavelength range from 180 nm to 1 mm primarily by the process of controlled stimulated emissionò. Laser light has two unique characteristics: a very narrow band width and a high level of coherence.

Laser light is generally considered to be visible and collimated, *i.e.* travelling in a long, straight line. This is true for many lasers: the most well-known collimated laser is the laser pointer. However, medical lasers are generally neither collimated nor visible to the naked eye. In surgery, as with the carbon dioxide laser (10600 nm), the beam can be either focused for cutting or defocused for tissue ablation. Today lasers are widely used, even in domestic appliances and are basic components of modern technology. In medicine, lasers have been applied for decades in such diverse fields as surgery, ophthalmology and blasting of kidney stones.

In physics, coherence is a property of waves that enables stationary (i.e. temporally and spatially constant) interference. More generally, coherence describes all properties of the correlation between the physical quantities of a wave. Two waves can combine to create a larger wave (constructive interference) or detract from each other to create a smaller wave (destructive interference), depending on their relative phase. Two waves are said to be coherent if they have a constant relative phase (Figs.1,2). (http://en.wikipedia.org/wiki/Coherence_%28physics%29).

The degree of coherence is measured by the interference visibility, a measure of how perfectly the waves can cancel each other out by destructive interference. The beam may or may not be parallel and the intensity can vary from a fraction of a milliwatt to many watts. Coherence is reported to be important in biostimulation. It appears to have

an additional positive effect in laser surgery, but the main advantage of surgical lasers has little to do with the coherence.

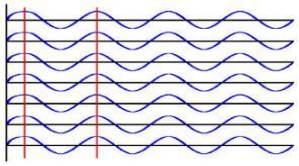

Figure 1. Coherent light

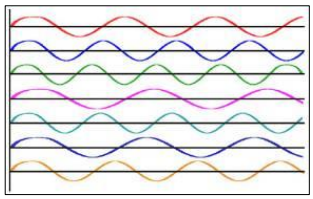

Figure 2. Incoherent light

The length of coherence varies considerably between different types of lasers. The shorter the bandwidth, the longer the length of coherence. The light from a gas-based laser such as the HeNe (632.8 nm), has a coherence length directly from the tube of many metres and a very narrow spectral bandwidth (Fig. 3). However, passage through an optic fibre reduces the length of coherence considerably. Diode lasers, such as the InGaAlP, can have a wavelength similar to the HeNe, but the length of coherence from a laser diode is considerably shorter.

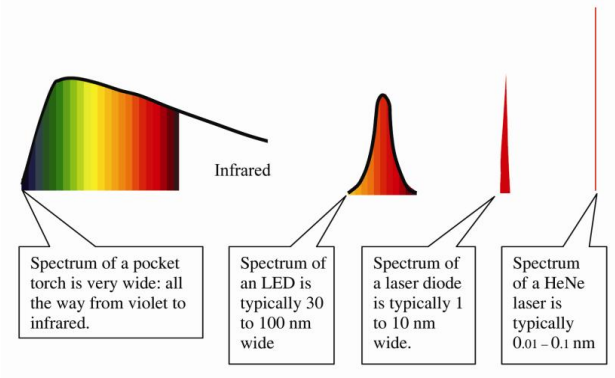

Figure 3. Spectral bandwidth of different light sources

From: Laser Therapy, Clinical Practice and Scientific Background. Prima Books AB, 2002

THERAPEUTIC LASERS

The first commercialised biostimulative laser was a HeNe laser of less than 1 mW. With its high degree of coherence the HeNe is an attractive laser for biostimulation but limited by the need for an optic fibre, the size of the machine and the still rather low power option, now typically in the range 5-25 mW. It has generally been replaced by the InGaAlP laser, a diode producing red laser in the range 600 - 700 nm and able to deliver as much as 500 mW. The most frequently used laser in dentistry is the GaAlAs laser. It often operates in the spectrum between 780 and 830 nm. The 808 nm diode dominates the market. Output is typically between 10 and 500 mW. An advantage of the diode lasers is the small size and option for battery operation, making them rather handy and portable. These lasers all work in continuous mode, but can be mechanically or electronically pulsed (ñchoppedò). The optical penetration of the light varies with several parameters. The short wavelengths in the red spectrum have less penetration than those in the infrared spectrum. The type of tissue also influences the penetration. Mucosa is rather transparent, bone and cartilage fairly transparent whereas penetration into muscles is poor, due to the thickness of the tissue and the high vascularisation. Blood is a major absorber of the light. Penetration also varies with distance from the laser source to the target tissue: contact irradiation forces the light into the tissue, while irradiation from a distance causes more reflection of the light.

The GaAs laser is different, being a superpulsed laser working at 904 nm. Superpulsed lasers produce very powerful, pulsed peaks in the Watt range, but the duration of the peak is typically only 200 nanoseconds. A GaAs laser presenting a Peak Power of 10 W typically has an average output of 10 mW. Pulsing is reported to be of importance in biostimulation, but the evidence to date is based entirely on *in vitro* studies (Karu, 2007). Little is known of the role of pulsing in clinical application.

Laser phototherapy (LPT) Mechanisms

To achieve an effect, the photon must be absorbed by photoreceptors. There are many photoreceptors in the human body, *e.g.* the porphyrins. However, the most important receptor has been identified as cytochrome c-oxidase, the terminal enzyme of the Krebôs cycle. Cytochrome c-oxidase is an ATP producer (Passarella *et al.* 1984, Pastore *et al.* 1996, Karu 2007). A cell in a reduced condition can be revitalized by stimulating production of ATP. The laser light in the red spectrum severs the bond between NO and cytochrome c-oxidase, allowing the enzyme to initiate production of ATP (Huang *et al.* 2010). This production in itself leads to a cascade of events, such as increased permeability of the cell wall and the $Ca^{2}+$ circulation. It has been speculated that infrared laser light bypasses this process and acts directly on the cell membrane permeability and the calcium ion channels. Cells in a normal redox situation are not particularly responsive to LPT: the best effect is seen in cells in a reduced redox situation (Almeida-Lopes *et al.* 2001). To date, studies of LPT have confirmed the effects as natural processes and no effects ñoutside the boxò have been reported.

THE ND:YAG LASER

This type of laser produces light in a single crystal of Yttrium-Aluminium-Garnet with the addition of - for example - elemental neodymium (Nd). The full name of this laser is thus Neodymium-Yttrium-Aluminium-Garnet. Normally the laser is pumped by a very strong flash lamp. A new type of Nd:YAG laser is the diode laser pumped YAG:laser, in which instead of a flash lamp, powerful GaAlAs lasers are used to pump optical energy to the Nd:YAG laser rod. The wavelength is 1064 nm. The light is distributed via optical fibres, typically 300-600 micrometers in diameter.

The pulses are always in the millijoule (mJ) range and both the number of pulses per second and the pulse length can be tailored by the operator to suit the intended target. Most Nd:YAG lasers do not have a water cooling system.

The Nd:YAG lasers are in the watt (W) range. For dental use they are always pulsed, each pulse providing a short energy in the millijoule range. The length of the pulse is measured in nanoseconds (ns). Thus, the actual energy at the tips is determined by several factors, such as basic output power, number of pulses per second and the pulse length. These are often pre-programmed on the laser but can be chosen individually to adapt to the situation or the experience of the operator. These parameters describe the energy applied: the dose (energy density) is also influenced by the size of the optical fibre. A thin fibre produces higher energy density at the tips: hence a 300 micron fibre has an energy density four times greater than that of a 600 micron tip. The use of water cooling will also influence the actual dose locally. Thus many parameters influence the actual energy delivered. In this context, the technique adopted by the operator is also an important determinant.

Modern dental Nd:YAG lasers are free-running and pulsed, in contrast to other continuous wave lasers with gated pulse options. The ablative capacity is set either by increasing the output power or the pulse repetition rate. The procedure is undertaken in tissue contact mode and in constant motion.

For pulsed lasers, peak powers are orders of magnitude higher than average powers. There are pronounced spikes, with peak power 1000 times higher than the average and relatively long rest periods. Pulse width (the duration of each pulse) varies from 90 to

1200 microseconds in different pulsed Nd:YAG lasers and is an important component of this technology. The number of pulses (frequency, pulse repetition rate) per second is one of the crucial variables in pulsed Nd:YAG lasers. With a high repetition rate from 10 to 100 Hz in different devices, smoother cutting can be achieved at a very low power setting, because the peak power in each pulse can be very high (White *et al.* 1994).

The 1064 nm wavelength is invisible, which complicates objective evaluation of the actual effected area. Observation made by the author, using an infra-red camera has revealed that the light is not concentrated around the fibre tip, but is spread like a small sphere over a rather large area.

The mechanisms underlying the Nd:YAG (surgical) laser

Nd:YAG laser energy is absorbed by tissue and it is this absorbance that allows surgical excision and coagulation of tissue (Goldstein *et al.* 1995). Absorption by different dental tissues is illustrated in Figure 5: absorption by hydroxyapatite is moderate. At this wavelength, the ablative effect on hard dental tissue is obviously rather low. This wavelength has a particular affinity for melanin or other dark pigments. Therefore dark-pigmented microbes are more sensitive to this laser and can be eliminated at quite low power settings, with no collateral damage to the adjacent tissue. The choice of wavelength is important to reach a bactericidal effect. Harris & Yessik (2004) developed a method for quantifying the efficacy of ablation of *Porphyromonas gingivalis* (Pg) *in vitro* for two different lasers. The ablation thresholds for the two lasers were compared in the following manner: Pg were cultured on blood agar plates under standard anaerobic conditions. Haemoglobin is a primary absorber of the wavelengths tested: thus in this context the blood agar simulated gingival tissue. Single pulses of laser energy were delivered to the Pg colonies and the energy density was increased until a small smoke plume was observed coincident with a laser pulse. The energy density at this point was denoted as the ablation threshold. Ablation thresholds to a single pulse were determined for Pg and for blood agar alone.

The investigation showed a major difference in ablation thresholds between the pigmented pathogen and the host matrix for pulsed Nd:YAG, representing a significant therapeutic window. Pg could be ablated without visible effect on the blood agar.

An 810 nm diode laser, on the other hand, destroyed both the pathogen and the gel. Clinically, the pulsed Nd:YAG may selectively destroy pigmented pathogens, leaving the surrounding tissue intact. The 810 nm diode laser may not demonstrate this selectivity due to its longer pulse length and greater absorption by haemoglobin (Harris & Yessik 2004).

It is postulated that the Nd:YAG laser eliminates primarily the dark-pigmented microbes, such as Pg, whereas *Aggregatibacter actinomycetemcomitans* (Aa) which

has no pigments, would not be similarly reduced. However, in a study by Andrade *et al.* (2008) Aa was completely eliminated directly after irradiation, but had regained approximately 50% of baseline level after 6 weeks. Such recurrence is reported in several studies and is attributed to cross contamination from non-treated pockets and/or saliva (Teughels *et al.* 2000).

The Nd:YAG laser has a certain biostimulative effect and this contributes to the enhanced postoperative healing after Nd:YAG laser surgery. The energy densities in the most peripheral zone (LPT) fall within the biostimulative range, as illustrated in figure 4**.**

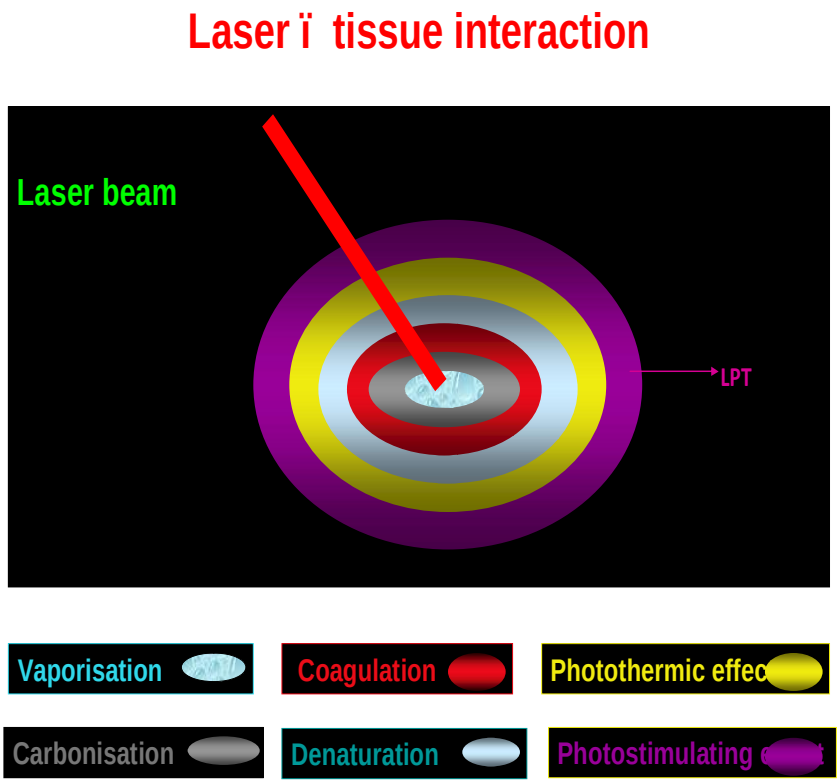

Figure 4. Schematic illustration of the different light intensity zones (surgical lasers) From: The New Laser Therapy Handbook, Prima Books AB, Grängesberg, 2010. Courtesy: Edson Nagib

Negative thermal effects of Nd:YAG laser have been reported from *in vitro* studies (Liu *et al.* 1999, Israel *et al.* 1997). However, *in vivo*, effects on the root surface and the pulp are not well-documented (Gaspirc 2001; Schwarz *et al.* 2008). The effect of laser irradiation on the surrounding tissues is influenced by parameters such as power, pulsing, fibre size, fibre angulations and cooling/no cooling. A study by White (1994) suggested that powers between 0.3ï 3.0 W should not cause a damaging rise in intrapulpal temperature. Likewise**,** Gold and Vilardi (1994) and Spencer (1996) also reported that use of laser at 4 W is safe and does not damage the root surface.

Nd:YAG, which has little absorption in water, may be effectively cooled with simultaneous air and water spray. Lasers with limited transmission through enamel and dentine may also be effectively cooled by an air and water spray immediately after lasing. Several studies have confirmed that application of an air and water spray provides adequate heat protection to the pulp, comparable with cooling of the conventional rotary bur (Miserendino *et al*. 1994). The absorption in different dental tissues is illustrated graphically in figure 5.

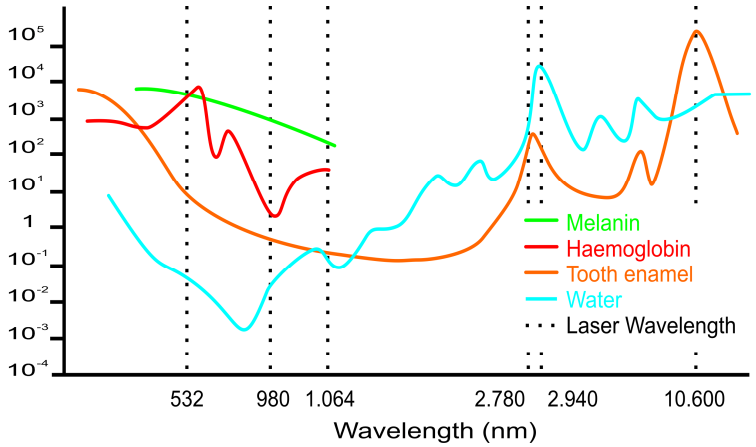

Figure 5. The absorption spectrum for melanin, haemoglobin, enamel and water.

HISTORY OF MEDICAL AND DENTAL APPLICATIONS OF LASERS

The first laser to be used in medicine was a ruby laser (wavelength 694 nm) and it was soon applied in surgical procedures. The ruby is a solid state laser with a ruby rod as the lasering medium. The first gas laser for surgery was the carbon dioxide (CO_2) laser. It had several appealing features in that it was able to remove superficial tissue without harming the underlying tissues, due to the very high absorption of the 10600 nm in water. Although this laser was expensive and large, it was soon accepted as a useful tool in dental surgery, performing tissue ablation with a good degree of coagulation. Conditions such as haemangiomas, leukoplakias and fibromas could easily be ablated and malignancies could be removed surgically by focusing the beam. One of the first Scandinavian papers on this topic was an animal study published by Luomanen (1987).

The Nd:YAG laser was also readily adopted in medicine, especially in the field of ophthalmology. With a wavelength of 1064 nm, this laser could coagulate ocular bleeding in diabetics, among other things. Myers (1991) was the first to apply the Nd:YAG laser in dentistry: in fact, the first laser tested belonged to Myersô brother, an ophthalmologist. This laser proved useful for minor dental surgery, with a good coagulatory effect. An unexpected observation was that little or no analgesia was required. The laser could also be used to ñnumbò a tooth before drilling. Application as a substitute for the ñdental drillò attracted much public attention, but was not a great success. To be absorbed into the dental hard tissues, a dark dye had to be applied to the tooth before ñdrillingò and the process was very slow. It was not until the advent of the Er:YAG lasers in the late 1990s that application of lasers for removal of hard dental tissue became more widely adopted. These versatile lasers can penetrate dental hard tissue at almost the same rate as a high-speed turbine drill. A major advantage is that little or no analgesia is necessary. Laser-based methods have also been introduced as aids for detection of early carious lesions, such as quantitative light-induced laser fluorescence, using a diode laser with 655 nm (Tranaeus *et al.* 2005).

The most recent additions to the dental laser family are the diode lasers. These typically emit at wavelengths of 808, 940 or 980 nm, with outputs ranging from 3-7 watts. The light is transmitted through an optical fibre. They are commercialised for soft tissue management but are also used for endodontic decontamination and sulcular debridement (Romanos *et al.* 2004). The diode lasers are much smaller than Nd:YAG and Er:YAG lasers and less expensive.

Originally, the lasers introduced for medical application were all surgical in that they were able to cut, evaporate and coagulate. However, another application was reported very early by McGuff *et al.* (1965), studying the potential effect of the ruby laser on tumours in hamsters. Different doses of ruby laser light were applied to various tumours implanted in the animalsô cheek pouches. The results were unexpected: the hamsters receiving laser light lived longer and even recovered completely, while none of the control hamsters survived. The underlying mechanisms were not clarified and the published papers do not appear to have attracted much attention. However, the results were noted by the Hungarian surgeon Endre Mester (1967), who undertook some basic experiments with a ruby laser on mice. The fur was shaved and wounds were created bilaterally (Fig. 6). One side was irradiated with low doses of ruby laser and the other side served as the control. Initially it was intended to increase the dose gradually, but it was soon discovered that the irradiated wounds healed faster than the non-irradiated wounds, while at higher doses the irradiation inhibited the wound healing. Even the shaved fur grew back more quickly on the irradiated side. This was the first documentation of the phenomenon of ñbiostimulationò. These lasers have then been applied for a great variety of indications, such as radiation induced mucositis (Bensadoun *et al.* 1999) and paresthesias of the inferior alveolar nerve (Khullar *et al.* 1996).

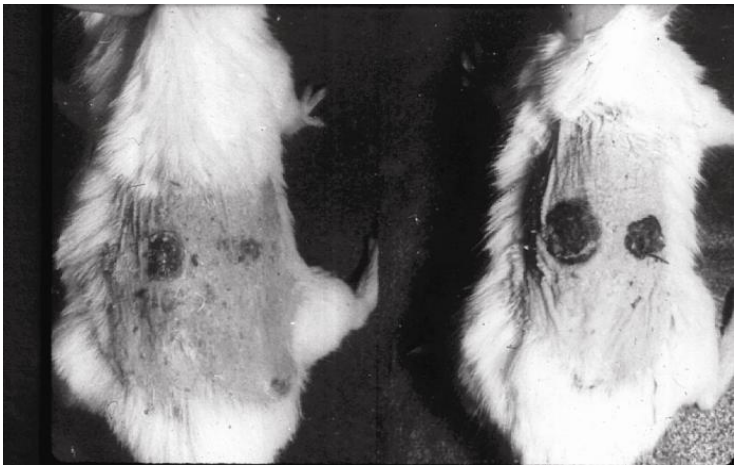

Figure 6. Dorsal wounds on mice treated with ruby laser on the right side only

From: Laser Therapy, Clinical Practice and Scientific Background. Prima Books AB, 2002. Courtesy: Andrew Mester.

Safety and contraindications

The therapeutic lasers used in dentistry are classified as 3B, considered as low risk devices and according to Swedish authorities (Strålskyddsmyndigheten - SSM) may be used freely by anyone. Although the risk of eye injury is very low, protective goggles are nevertheless recommended for the patient. There is no harmful heating of the tissue when lasers are used in the recommended energy ranges. Since the limit of the ionising radiation is around 320 nm, there is no risk of cancer induction in tissues.

None of several alleged contraindications have been verified during 40 years of use. There are, however, some caveats. Due to the risk of stimulating malignant cells, laser irradiation should not be used over known malignancies. However, the use of the therapeutic laser is well documented for reducing the incidence of mucositis in patients receiving chemo- and radiation therapy. Laser treatment is also contraindicated in patients with coagulation disorders, because the effects of lasers on the mechanisms of coagulation have yet to be determined.

Dosage

To reach the dosage (also called fluence or energy density) the power of the laser must be known. The power is expressed in milliwatts (mW). The energy delivered is a function of the time. Thus, mW x seconds = energy. The energy is expressed in joules (J). For instance, a laser of 100 mW used for 10 seconds delivers 1000 mJ = 1 J.

The dose is a function of the size of the irradiated area, expressed in cm^2. For instance, if 1 J is applied to an area of 1 cm^2 the calculation is 1 J/1 cm^2 = 1 J/cm^2 (dose). However, if the irradiated area is 0.25 cm^2 the calculation is 1 J/0.25 cm^2 = 4 J/cm^2.

Another important factor in biostimulation is the power density, meaning the number of mW over an area. If the laser emits 100 mW over an area of 1 cm^2, the calculation is 100/1 = 100 mW/cm^2. If the area is only 0.25 cm^2 and receives the same number of mW, the calculation is 100/0.25 = 400 mW/cm^2. In laser phototherapy, it is important that all these variables are controlled, because each evokes different cellular reactions. In the field of dentistry, the expression power density is quite familiar, because the ñpowerò of the dental curing light is expressed in mW/cm^2.

LPT follows the Arndt-Schultz law, (Fig. 7) which stipulates that for every substance, small doses stimulate, moderate doses inhibit, and large doses destroy.

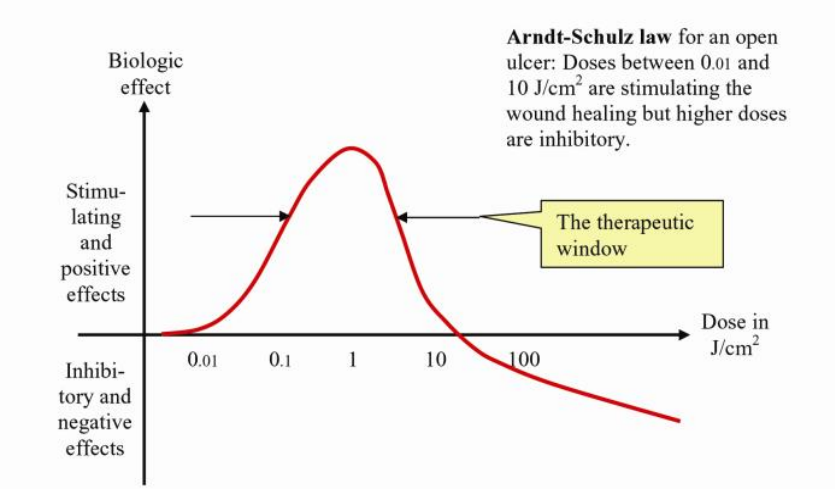

Figure 7. Arndt-Schultz law in phototherapy

From: Laser Therapy, Clinical Practice and Scientific Background. Prima Books AB, 2002

LASER PHOTOTHERAPY IN PERIODONTOLOGY

Inflammation

Local inflammation is the central process in gingivitis and periodontitis. Acute clinical manifestations include gingival swelling, redness and bleeding on probing. Inflammation is basically a functional reaction necessary to protect the body from bacterial invasion. Histologically an influx of leukocytes can be seen, primarily neutrophils and monocytes/macrophages. When the inflammation becomes more chronic the number of plasma cells and lymphocytes increases.

In the studies on which this thesis is based, clinical inflammation has been registered as the Gingival Index (Silness & Löe 1964). This index assesses a combination of swelling, redness and bleeding on probing. Changes in gingival pocket depth were also measured: initially these reflect changes in the inflammatory condition. To complement the clinical registration of inflammation, gingival crevicular fluid (GCF) volume has been measured. GCF is an exudate/transudate that continuously flows out of the gingival pocket. The volume increases with increasing inflammation and may thus be considered a surrogate marker of inflammation, that is more objective than clinical assessment of gingivitis (Golub & Kleinberg 1976).

To further assess the local inflammation a number of inflammatory mediators in GCF have been analysed. Interleukin-1ß (IL-1ß) is a proinflammatory cytokine that is released by many different cells, among them macrophages. IL-1ß can be considered a general marker of the severity of inflammation in the tissues (Dinarello 2005). MMP-8 is a collagenase produced and released by several cells but mainly by neutrophilic granulocytes during their migration from the blood capillaries to the inflamed tissues (Sorsa *et al.* 2004). MMP-8 can thus be seen as an expression of neutrophil influx and as such as a marker of inflammation. Elastase is a protease typical for polymorphonuclear leukocytes (PMNL). It is mainly released from the neutrophils during phagocytosis and may be regarded as an indicator of neutrophil activation (Janoff 1985). IL-8 is a chemokine and an important inflammatory mediator released from endothelial cells (Gamonal *et al.* 2000).

In some cases the basically protective inflammatory response becomes tissue destructive, i.e. periodontitis. The reasons for this change from a protective to a tissue degrading inflammation is unclear but a Gram Negative anaerobic microflora together with a susceptible host is probably necessary. The Swedish Council on Health Technology Assessment estimates that signs of periodontitis are present in more than 40% of the Swedish adult population. Hugoson & Norderyd (2008) reported a 13% incidence of severe periodontitis, although this is regional and age-related. Periodontitis is more pronounced in those above the age of 40 years. Some forms of periodontitis are very aggressive and may result in rapid loss of periodontal attachment and destruction of alveolar bone. A major characteristic of the disease is the presence of bacteria in the gingival pocket. Conventional therapy aims at reducing the bacterial load and suppressing inflammatory signs through mechanical or chemical intervention, sometimes including antibiotics. The outcome of mechanical treatment may be compromised by the presence of furcations, invaginations and concavities. In these cases there is a need for an additional treatment approach.

Periodontitis is primarily an inflammatory process which generally causes only minor pain or discomfort. Thus scaling and root planing (SRP) are undertaken in order to remove calculus and granulation tissue adhering to the root surface, and to create conditions which facilitate maintenance of good oral hygiene. While SRP is considered to be fundamental periodontal treatment, it is not always completely successful and adjuvant therapies have been suggested.

In this context, laser therapy has been proposed, the goal being to target the inflammation. However, to date the scientific basis for this treatment modality is not well documented. The optimal parameters for each laser and for each particular intervention have yet to be determined.

Therapeutic lasers

Studies using *therapeutic lasers* have reported an effect on inflammation, mainly by shortening the inflammatory process ï which in itself is essential for healing (Choi *et al.* 2005, Pejcic *et al*. 2010). Sawasaki *et al.* (2009) and Silveira *et al.* (2008) reported significantly increased mast cell degranulation after 670 nm laser irradiation of human mucosa and gingiva, respectively. The degranulation leads to a release of histamine and should theoretically stimulate an increased inflammatory response. It is speculated that the increased mast cell degranulation accelerates the inflammatory process, which eventually leads to wound healing via fibroblast proliferation and collagen synthesis.

Chronic periodontal inflammation leads to the destruction of the periodontal ligament and subsequently to loss of alveolar bone. The latter is mediated primarily by osteoclasts and triggered by the pro-inflammatory molecule Prostaglandin E_2 (PGE_2) (Choi *et al*. 2005). There is some evidence in the literature that patients receiving LPT in conjunction with conventional periodontal treatment experience improvement in clinical inflammation (Pejcic & Zivkvic 2007).

Although gingivectomy is not a common procedure in modern periodontal therapy, studies by Amorim *et al.* (2006) and Özcelik *et al*. (2008a) report improved healing associated with application of 685 and 588 nm irradiation, respectively.

Garcia *et al*. (2009) compared LPT as an adjuvant to SRP for treatment of induced periodontitis in rats. Treatment was compared to dexamethasone or saline solution. Radiographic and histometric analysis showed less bone loss in animals treated with SRP + LPT. A study by Pires de Oliveiro *et al.* (2008) has confirmed the stimulative effect of LPT on osteoblasts. Özcelik (2008) has reported positive effects of LPT in treating intra-bony defects with EMD ï enamel matrix protein derivate.

Periodontal wound healing is an important phase when the composition and integrity of periodontal structures have been threatened by gingivitis, periodontitis or trauma. The restoration of fibrous attachment and lost bone requires regeneration of destroyed connective tissue, formation of new cementum and bone and attachment of new

connective tissue fibres (Aukhil 1992). Thus successful repair involves several processes, including inflammation and cellular migration, proliferation and differentiation (Pitaru *et al.* 1994, Loevschall & Arnholt-Bindslev 1994).

Several *in vitro* studies have shown that LPT at certain wavelengths may stimulate fibroblast proliferation, provided that certain combinations of exposure parameters and power densities are used (Yu *et al.* 1994, Almeida-Lopes *et al.* 2001, Pereira *et al.* 2002, Azevedo *et al.* 2006). At higher energy densities, no effect or even decreased proliferation has been reported (Kreisler *et al.* 2003). Therefore, Karu (1990) suggested a ñwindow-specificityò at certain wavelengths and energy densities, for which a positive effect of laser phototherapy can be expected.

An important aspect of laser-tissue interaction is the coherence of the laser light. Many studies have compared the biological effect of coherent and incoherent light and to date all studies indicate a superior effect by lasers producing a long length of coherence. Generally the comparisons have been made between lasers and Light Emitting Diodes (LED). These light sources have a spectral width of 30-100 nm, while the spectral widths of the lasers are in the range 0.01 ï 1 nm. A study by Rosner *et al.* (1993) investigated the effect of HeNe laser on regeneration of crushed optical nerves. While HeNe laser delayed the degenerative process, non-coherent infrared light was ineffective or affected the injured nerves adversely.

Coherence seems to be an important parameter in light stimulation of biological scattering in bulk tissue. Karu *et al.* (1982, 1983) studied the importance of different light characteristics in cell stimulation, such as wavelength, coherence, dose and time regimens and concluded that coherence had no effect. However, in this context it is important to note that these studies were conducted *in vitro* on monolayers of cells: the cells were directly exposed to the laser and there was no scattering in the medium. As the laboratory conditions do not simulate the clinical setting, the results should be extrapolated with caution.

Nd:YAG laser

Nd:YAG lasers have been used in periodontal treatment for many years but consensus has yet to be reached about the general efficacy or the specific efficacy of different power settings and clinical techniques. An important part of the laser device, which is rarely discussed, is the optical fibre. Most bare fibres consist of a glass rod core made of silica quartz with an outer surface cladding of different refractive index, and an outer protective vinyl jacket. The standard options are diameters ranging from 200 to 600 micrometers. As the fibre diameter decreases, the energy densities increase and fibre flexibility increases. Thin fibres are popular because of the high power density but less than ideal for closed curettage, because they are prone to fracture and the energy density is too high. The energy density of a 300 micrometer fibre is four times as high as that of a 600 micrometer fibre. Thus, the use of a thin fibre in a closed area has disadvantages. The high power densities will char areas in the pocket and carbonized tissue will adhere to the tip. In the dark carbonized areas, absorption of the light increases and so does heat. The aim of the laser treatment is not to use the instrument for cautery, but to take advantage of the interaction between the light and the specific tissue irradiated. Further to that, a thicker diameter makes the fibre stronger and difficult-to-reach areas can be accessed more readily.

A major advantage of Nd:YAG laser periodontal therapy is that the procedure is relatively pain free. From the patientôs perspective this is certainly a major advantage. The degree of pain is largely determined by the skill of the operator. However, in some cases an analgesic gel or spray is advisable during the initial phase of the surgery. After a while, it seems that the laser in itself provides an anaesthetic effect. Sulcular debridement around hypersensitive teeth may sometimes be painful. In these cases, the tooth crown can be irradiated from a short distance without water until an anesthetic effect of the pulp is achieved. For the same reason, no water should be used when hypersensitive tooth necks are treated with Nd:YAG laser. In combination with water the area will be cleaned and the tubuli even more open. Without water there is the potential for the laser to seal the tubuli (Lan & Liu 1996).

In general it can be stated that correctly applied, the lasers themselves are not dangerous or damaging. It is the lack of knowledge that creates damage. The undesirable side effects can vary primarily with power and energy density and secondly with the type of laser used.

AIMS

GENERAL AIMS OF THE THESIS

Several potential roles have been proposed for laser application in periodontal treatment but the reported outcomes of studies to date are contradictory. The available data are inadequate for recommendations with respect to optimal laser treatment parameters.

The present thesis is based on a series of clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. The studies were undertaken with the overall aim of evaluating the potential of adjunctive application of therapeutic and surgical lasers to improve the short and long-term treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters. Such studies are essential in order to provide evidence on which to base recommendations for clinical application.

Four studies were undertaken, the first two on therapeutic lasers and the third and fourth studies on the Nd:YAG (surgical) laser.

SPECIFIC AIMS

The specific aims of the four studies were as follows:

Study I: to examine the effects of irradiation with laser phototherapy on inflamed gingival tissue

Study II: to determine the possible influence of the length of coherence in laser phototherapy

Study III: to compare the outcome of treatment of periodontitis by combined SRP and a single application of water-cooled Nd:YAG laser irradiation with that of SRP alone

Study IV: a follow-up study of Study III, to determine whether the positive advantages of the laser treatment were sustained over a longer time period

MATERIAL AND METHODS

The following is a brief description of the materials and methods used in the four studies. Detailed descriptions of the material and methods are presented in the original papers (I-IV).

Periodontal Examination

Periodontal evaluation included PI (Plaque Index, Löe 1967) and GI (Gingival Index, Silness & Löe 1964). PPD (Probing Pocket Depth) was measured with a graded periodontal probe (PerioWise, Premier Dental, Plymouth Meeting, PA, USA) at 4 sites (mesial, distal, buccal and lingual). In studies I and II, the maxillary teeth, from 17 to 13 and 27 to 23 were registered. In studies III and IV, all the mandibular teeth, except for the third molars, were registered.

Microbiological Examination

Subgingival plaque was harvested from the same site as GCF samples, by inserting sterile paper points (size 30) for 30 seconds. The paper points from each side were then pooled in sterile transport vials and sent to the laboratory for analysis. The subgingival microbiota was analysed using a checkerboard DNA-DNA hybridization method (Papapanu *et al.* 1997) and the frequencies of positive sites and of sites with cfu Ó10^6 were recorded. The following 12 micro-organisms were analysed: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythensis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Treponema denticola, Peptostreptococus micros, Selenomonas noxia* and *Streptococcus intermedia*.

Gingival Crevicular Fluid (GCF)

In all subjects, two GCF samples were taken from each side of the maxilla, after removal of supragingval plaque from the site to be sampled. The sites were isolated with cotton rolls and gently dried with an air syringe before sampling. To collect GCF, prefabricated paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA) were inserted until resistance was felt and removed after 30 seconds. GCF volume was measured with a calibrated Periotron 8000 (Oraflow Inc). Samples were pooled and

diluted in phosphate buffered saline (PBS) up to 1 ml. After elution for 15 minutes, the strips were removed and the samples frozen at -20°C.

Laboratory analyses

Studies I and II

IL-1ß

The IL-1ß content of the GCF samples was measured with sandwich ELISA, using a monoclonal antibody (MAB 601, R&D Systems, Minneapolis, MN, USA) diluted 125 times in carbonate buffer, coated onto microtitre plates (Nunc Maxisorb Nanc A/S Roskilde, Denmark) overnight at + 4ºC. The plates were blocked with 1 % human serum albumin (HAS) for 1 hour in room temperature. The detection antibody (BAF 201, R&D Systems), a biotinylated polyclonal goat antibody diluted 250 times, was incubated for 45 min at 37°C. After washing, horseradish peroxidase conjugated streptavidine, diluted 200 times in PBS +0.1% HSA, was added to the plates and incubated in the same way as for the detection antibody.

The plates were washed again and the undiluted substrate (TMB, Sigma Chemical, St. Louis, MO, USA) added. The reaction was stopped with 1M H_2SO_4 after 15 minutes. Absorbency was read at 450 nm in a spectrophotometer (Millenia Kinetic Analyser, Diagnostic Product Corporation, Los Angeles, CA, USA).

Elastase Activity

Total elastase activity was measured with a chromogenic substrate specific for granulocyte elastase (Tanaka *et al.* 1990), (L-pyroglutamyl-L-propyl-L-valine-p-nitroaniline, mw 445.5 Da, on a 96-well microtitre plate (Nunc Maxisorb, Nanc A/S). After 2 h of incubation at 37ºC, absorbency was read for a second time. The total elastase activity is expressed in mAbs (milliabsorbances).

MMP-8 & IL-8

MMP-8 & IL-8 were analysed with commercial kits (Quantikine ®, R&D Systems Inc.) in accordance with the manufacturerôs instructions. A monoclonal antibody specific for MMP-8 had been pre-coated on to a microplate. Samples diluted 10 times

were pipetted into the wells and incubated at room temperature for 2 h. The plates were then washed and a monoclonal antibody against MMP-8, conjugated to horseradish peroxidase, was added and incubated again, as described previously. After another washing procedure, the substrate solution was added and the reaction stopped after 15 min. with a stop solution. Within 20 min., the absorbency at 450 nm was read in a spectrophotometer. The MMP-8 was expressed in ng and the amount of IL-8 in pg.

Study III

IL-1ß, 4, 6, 8 and MMP-8

IL-1ß, IL-4, IL-6 and IL-8 were analysed with Multiplex bead kits, using a Luminex 100 (Luminex Corp., Austin, TX, USA) and commercial immunoassays, Lincoplex high-sensitivity human cytokine panel (Lincoplex/Millipore, St. Charles, MO, USA) according to the manufacturerôs instructions. The result was calculated with Bio-Plex Manager software (Bio-Rad Laboratories, Hercules, CA, USA) and the cytokine levels were determined as the total amount per site (pg) in the fluid. The collagenase MMP-8 was similarly analysed, but with a kit from R&D Systems (Abingdon, UK).

Radiographs

Digital bite-wing radiographs (Siemens, Bensheim, Germany) were taken with the vertical long axis of the hemi-mandible using a software programme (Schick Technologies Inc., NY, USA).

In Study IV all radiographs were taken by the author. Two observers recorded baseline and postï operative mandibular alveolar bone levels, in millimetres, at all approximal surfaces, from the mesial of the second molar to the distal of the canine. Alveolar bone loss was measured from the cemento-enamel junction (CEJ) to the most apical portion of the alveolar bone. Teeth with suspected or obvious carious lesions at the CEJ were not included.

Statistical methods

In studies I & II, statistical analyses were performed using Statistica 7 (Statsoft Inc. , 2005, Tulsa, USA).

In Study I, the significance of the differences in treatment effect between placebo and laser was calculated with the Student paired t-test or the Wilcoxon signed rank test. The frequencies of positive subjects and of subjects with Ó10^6 cfu of the analysed bacteria were calculated with Fisher's exact test.

In Study II, the significance of the differences in treatment effect between the two lasers was calculated with the Wilcoxon signed rank test.

In studies III and IV statistical analyses were performed using Statistica v.6.0 (Statsoft Inc. , 2005, Tulsa, USA).

In Study III, changes in the clinical parameters from baseline to follow-up, and between the treatment modalities, were assessed for statistical significance using a paired t-test. The laboratory data were analysed using the Wilcoxon signed rank test. Significance was set at $p<0.05$.

In Study IV, the paired t test was applied to assess the changes in clinical parameters from baseline to follow-up and between the treatment modalities. Normality was tested with the Kolmogorov-Simirnov test.

THE LASERS USED

Study I

A hand held, battery-operated Combi laser (Lasotronic AG, Baar, Switzerland) was used. The device has two wave lengths that can be used together or separately. In this study the wave lengths were utilized separately. Two lasers of identical appearance were used in the study: (Fig. 8) one active and one placebo, the latter having only a weak red LED diode instead of laser power. The active laser had two wavelengths, 635 and 808 nm, respectively. The output at 635 was 10 mW and at 808 nm 70 mW.

Figure 8. Active and placebo lasers

Study II

The lasers used in this study were a 3 mW HeNe laser 632.8 nm from Irradia AB, Stockholm, Sweden and a Pocket Therapy diode laser, nominally 650 nm, from Lasotronic AG, Baar, Switzerland (Fig. 9). Both had equal power of 3 mW.

Figure 9. The HeNe and the diode laser

Studies III and IV

The laser used in Study III and IV was a Nd:YAG (Genius 9 SLD) laser, emitting pulsed light 1064 nm, a fixed pulse repetition rate of 50 Hz , output from 1 W to 12 W and coolant water and air levels available from 1 to 15. The fibre diameter was 600 micron (Genius Dental A/S, Tureby, Denmark).

Summary of the four studies

I	Clinical study, double blinded Split mouth Clinical, immunological and bacteriological outcome	Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, MMP-8, IL-1ß, elastase, 12 bacterial species
II	Clinical study Split mouth, double blinded Clinical, immunological and bacteriological outcome	Plaque Index, Gingival Index, Pocket Depth, Gingival CrevicularFluid, MMP-8, IL-8, elastase, 12 bacterial species
III	Clinical study, single blinded, Split mouth Clinical and immunological outcome	Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, MMP-8, IL-1ß, IL-4, IL-6, IL-8.
IV	Clinical study, single blinded, Split mouth Radiological outcome	Plaque Index, Gingival Index, Pocket Depth, Gingival Crevicular Fluid, marginal bone loss

TREATMENT METHODS

Ethical Approval

These studies were approved by the regional ethical review board in Stockholm, Sweden. All subjects gave their written informed consent before inclusion.

Study I

Seventeen patients with moderate periodontitis were included, 10 women and 7 men. After clinical examination, all teeth were scaled and root planed (SRP). Oral hygiene instructions were given and controlled at each session. Baseline measurements were: Pocket Depth, Gingival Index and Plaque Index, all recorded before SRP. One week after SRP, samples of gingival crevicular fluid (GCF) and subgingival plaque were collected.

The laser therapy started one week later and continued once a week for 6 weeks. One side of the upper jaw was treated with the active laser and the other with the placebo unit.

The treated areas were:

(1) the buccal papillae, with 635 nm for 90 seconds (0.9 Joule, 4.5 J/cm^2, 50 mW/cm^2)

(2) 6 mm further apically, with 830 nm for 25 seconds (1. 75 Joules, 8.75 J/cm^2, 350 mW/cm^2)

(3) The sites were irradiated from both buccal and lingual aspects.

After the 6th week, the subjects underwent clinical re-examination, and new GCF and plaque samples were collected.

Study II

The study sample comprised twenty patients with moderate periodontitis. After clinical examination, all teeth were scaled and root planed (SRP). The dental hygienist now started the laser therapy, once a week for 6 weeks. One side of the maxilla was treated with HeNe laser and the other with a diode laser: choice of laser was determined by the toss of a coin. Each dental papilla on the teeth 13, 14, 15, 16, 23, 24, 25 and 26 was irradiated from the buccal aspect and 16 and 26 were also irradiated from the lingual

aspect. All irradiated sites received 0.54 J of energy per session, total energy per quadrant 3.25 J.

Studies III & IV

SRP + laser (SRPL) were used on one side of the mandible and the other was treated by SRP alone. Thirty patients (13 males and 17 females) with a mean age of 50 years (range 26 to 70 years) were originally included and randomly assigned to left or right side. The treatment outcome was evaluated after 3 months.

The laser used in this study was a Genius 9 SLD Nd:YAG (Genius Dental A/S, Tureby, Denmark), emitting pulsed light at a wavelength of 1064 nm. To avoid a thermal effect while maintaining optimal therapeutic effect, the instrument was set at level-five, giving the following parameters: average output 4 watt (W), energy per pulse 80 millijoule (mJ), pulse width 350 microseconds (µs), pulse repetition rate 50 Hertz (Hz), pulse peak power 240 W, average power density at fibre end 1430 W/cm^2 and peak power density 85800 W/cm^2. Laser energy per treated tooth was 240 ï 480 joules (J). The fibre diameter was 600 µm (0.002826 cm^2). Water and air cooling were used during irradiation. The time spent on each tooth varied between 60 to 120 seconds, depending on accessibility.

The fibre was held in constant motion, in contact with the pocket epithelial lining almost parallel to the long axis of the root. The power density and peak power density reported above are calculated by a hypothetical 100% emission through the small fibre tip. However, the energy is not emitted solely from the tip of the fibre; there is also considerable lateral emission. Due to the high uncertainty about the total area of tissue irradiated, the energy density (J/cm^2) was not calculated.

RESULTS

None of the participants reported any adverse side effects that could be related to the laser irradiation.

Study I

The results were as follows:

All clinical variables (PPD, PI, GI) showed greater reduction on the laser side ($p<0.02$). The GCF volume decreased more on the laser side, -0.15 µl, compared to the placebo side, -0.05 µl ($p<0.02$).

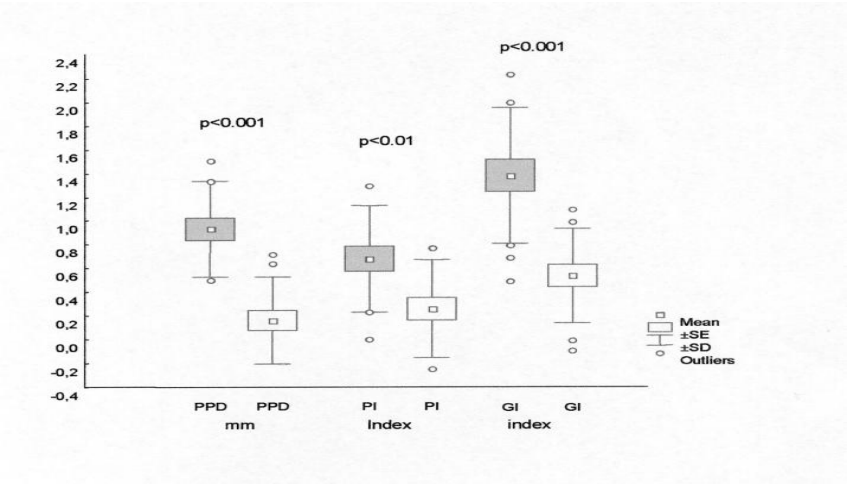

Figure 10. Box plot (above) shows the reduction in the clinical variables probing pocket depth (PPD), plaque index (PI) and gingival index (GI) after SRP and an additional treatment with laser or placebo. Filled boxes indicate the laser side.

Table 1. Change in GCF volume (mean SD) and the laboratory variables (median range) elastase activity, total amount of IL-1ß and MMP-8 in samples taken before and after treatment with laser or placebo, n=17 patients

	GCF Volume µl	**Elastase activity mAbs**	**IL-1ß pg**	**MMP-8 pg**
Placebo	-0.05	-9 (-576 - 252)	-1.7 (57.9 - 24.7)	90 ((8180 - 5859)
Laser	-0.15	32 (23 to 160)	-0.8 (24.4 - 82.8)	-70 (510 - 1145)
P-value	0.015*	0.15**	0.45**	0.052**

* p ï value calculated with the Studentôs paired t-test

** p-value calculated with Wilcoxonôs signed ï rank test.

The concentration of MMP-8 increased on the placebo side and was somewhat reduced on the laser side. The difference in treatment effect did not quite reach statistical significance (p=0.052). No differences were disclosed between laser and placebo sides with respect to elastase activity, IL-1ϐ concentration or microbiological analyses.

Study II

All clinical variables (PPD, PI, GI) showed greater reduction on the HeNe side (p-value = 0.001).

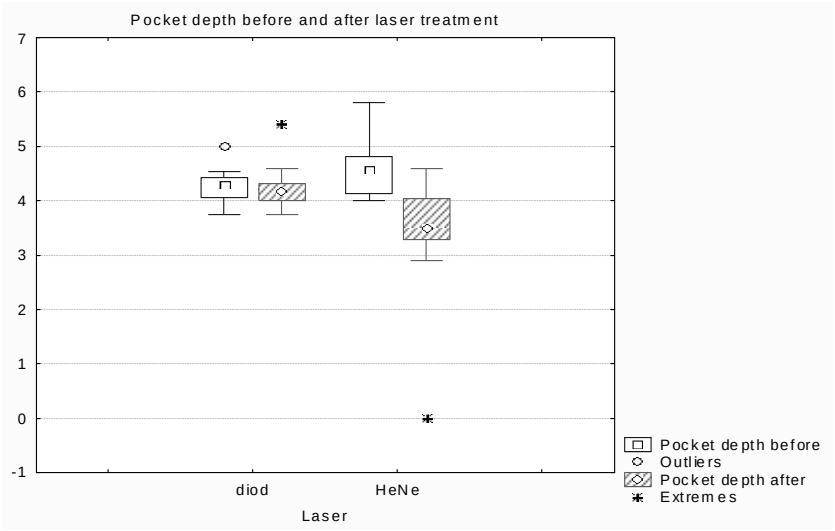

Figure 11. Box plot showing the reduction in the clinical variable probing pocket depth after SRP and an additional treatment with HeNe or diode lasers. Filled boxes indicate post treatment registrations.

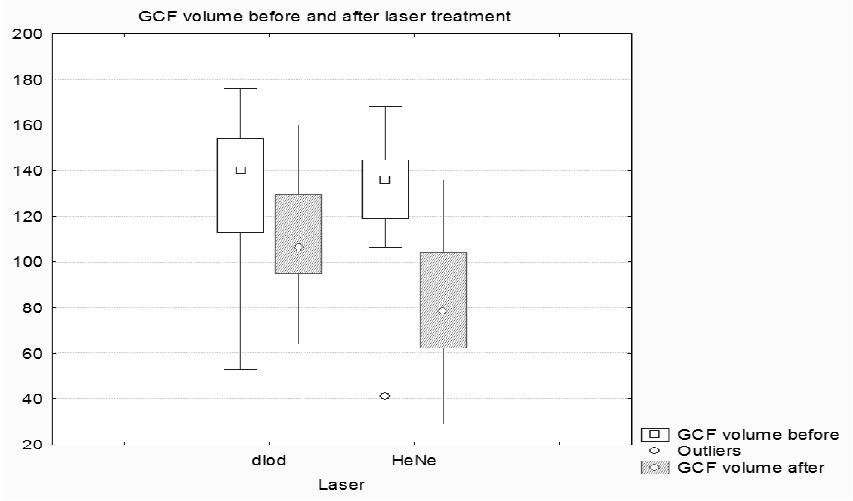

Figure 12. Box plot showing the reduction in GCF volume after SRP and an additional treatment with HeNe or diode lasers. Filled boxes indicate post treatment registrations.

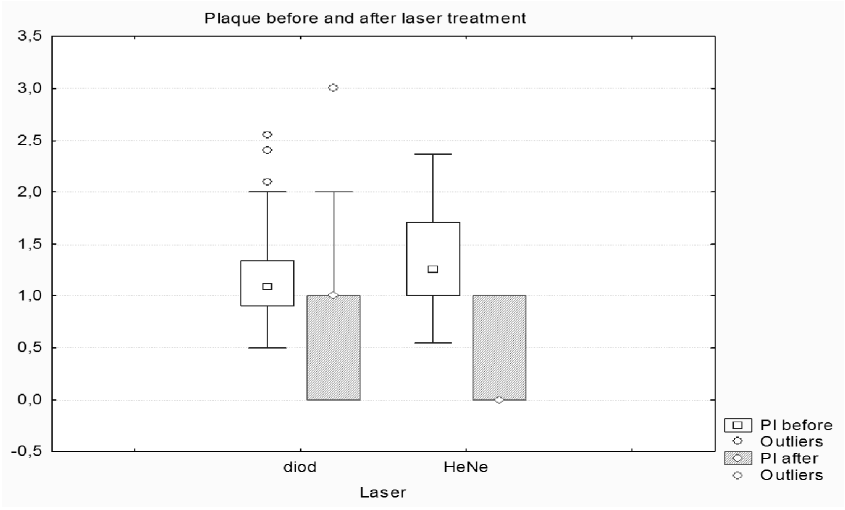

Figure 13. Box plot showing the clinical variables plaque index (PI), before and after SRP and an additional treatment with HeNe or diode laser. Filled boxes indicate post-treatment registrations.

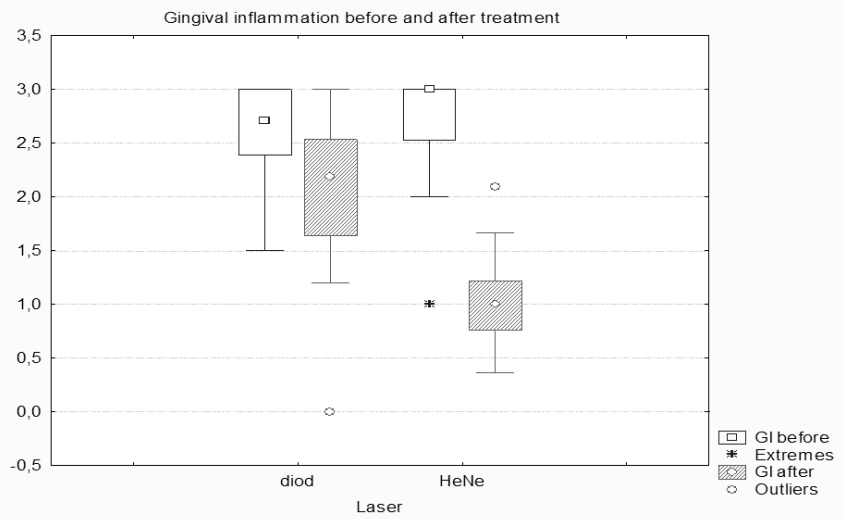

Figure 14. Box plot showing the clinical variable gingival index (GI), before and after SRP and an additional treatment with HeNe or diode laser. Filled boxes indicate post-treatment registrations.

Study III

Clinical outcomes

One week post-treatment, the PI ($p<0.05$), PPD ($p<0.001$) and GCF volumes ($p<0.001$) on the irradiated side had decreased significantly compared to the control side. The GI also decreased at the test side but the difference did not reach significance (Table 1). The three-month follow-up confirmed that the improvements were sustained. The treatment outcomes for the test side had improved significantly compared to the control-site (PPD [$p<0.01$], GI [$p<0.01$], PI [$p<0.01$] and GCF volume [$p<0.05$]) (Table 2). During the three-month follow-up, the mean PPD decreased by 0.6 mm on the test side compared to the control side.

Table 2. Clinical parameters (mean ± SD) in 30 patients with periodontitis. Change 1 indicates changes from baseline to one week follow-up and Change 2 from baseline to three months.

	Scaling and root planing (SRP)					Scaling and root planing (SRP) + laser irradiation				
Variable	**Baseline**	**1 week**	**Change 1**	**3 months**	**Change 2**	**Baseline**	**1 week**	**Change 1**	**3 months**	**Change 2**
Pocket depth (mm)	4.41 (0.27)	3.88 (0.42)	-0.53 (0.30)	3.57 (0.48)	-0.84 (0.39)	4.59 (0.44) p=0.012	3.61 (0.48) p=0.004	-0.98 (0.44) p=0.001	3.12 (0.60) p=0.001	-1.47 (0.46) p=0.001
Plaque index	1.96 (0.68)	1.26 (0.76)	-0.70 (0.59)	1.48 (0.57)	-0.48 (0.69)	2.02 (0.65)	1.05 (0.70) *P*<0.05	-0.97 (0.67) *P*<0.05	1.11 (0.69) *P*<0.01	-0.91 (0.81) *P*<0.01
Gingival index	1.97 (0.53)	1.46 (0.54)	-0.51 (0.58)	1.53 (0.54)	-0.43 (0.55)	2.11 (0.65)	1.40 (0.58)	-0.72 (0.50) *P*=0.07	1.10 (0.60) *P*<0.001	-1.02 (0.76) *P*<0.01
GCF volume (µl)	1.40 (0.31)	1.53 (0.34)	0.13 (0.36)	1.26 (0.41)	-0.14 (0.45)	1.44 (0.38)	1.12 (0.40) *P*<0.001	-0.32 (0.47) *P*<0.001	1.04 (0.41) *P*<0.05	-0.40 (0.47) *P*<0.05

p-values show the significance of the differences between the two groups, calculated with the paired t-test.

Table 3. Levels (median and interquartile range) of cytokines in pooled GCF samples (n=30). Change 1 indicates change from baseline to one week. Change 2 indicates change from baseline to three months.

	SRP alone					SRP+laser				
Cytokines (pg)	Baseline	1 week After treatment	Change 1	3 months After treatment	Change 2	Baseline	1 week After treatment	Change 1	3 months after treatment	Change 2
IL-1ß	0.32(0.89)	0.42(0.84)	0.02(0.48)	0.18(0.33)	-0.20(0.78)	0.46(1.35)	0.24 (0.71)	-0.26 (1.66) p<0.05	0.12 (0.71)	-.08 (0.77)
IL-4	0.66(2.04)	0.21 (1.26)	-0.30(1.07)	0.23 (2.01)	-0.09(0.689)	0.31 (2.81)	0.54 (2.94)	-0.06 (0.33)	0.03 (2.17)	-0.17 (0.31)
IL-6	0.08 (0.49)	0.0(0.31)	0.0(0.32)	0.0 (0.08)	0.0 (0.40)	0.10 (0.56)	0.0 (0.70)	0.0 (0.43)	0.0 (0.20)	0.0 (0.38)
IL-8	84.6 (80.8)	89.0 (86.9)	-5.4 (41.6)	59.0 (85.2)	-14.7 (76.6)	100.0(95.8)	44.6 (74.9)	-33.0 (100.9)	45.6 (81.4)	-28. 7(53.9)
MMP-8	7.00(29.5)	9.60 (33.2)	1.56 (8.4)	5.70 (14.0)	-1.89 (31.4)	12.9(37.4) p<0.05	6.91 (29.4)	-5.6 (23.9) p<0.05	2.70(14.8)	-4.88 (34.9)

P-values indicate significance of difference between the two treatment regimes (SRP compared to SRP plus Nd:YAG Laser

Study IV

Clinical and radiological results: At the follow up examination, PI ($p<0.01$), GI ($p<0.01$) and PPD ($p<0.001$) were significantly lower on the test side than on the control side. Radiological results showed a significant reduction in marginal bone loss on the test side compared to the control side ($p<0.05$).

Gingival crevicular fluid volume: GCF volume was significantly lower on the test side (mean change: -0.57 µl, range: -0.4 µl to 1.68 µl) than on the control side (mean change: 0.15 µl, range: -0.12 µl to 1.11 µl) ($p<0.01$). These results are summarized in Table 4 : clinical and laboratory outcomes.

Table 4. Summary of clinical changes in the control- and test-sites. *p*-values were calculated using paired t-test

	Control-site (SRP alone)			Test-site (SRP with Nd:YAG laser)		
Periodontal Variables	**Base-line (mean ± SD)**	**20-months follow-up (mean ± SD)**	**Change (mean ± SD)**	**Base-line (mean ± SD)**	**20-months follow-up (mean ± SD)**	**Change (mean ± SD)**
Probing pocket depth	4.41 ± 0.31	3.86 ± 0.76	-0.55 ± 0.60 †	4.58 ± 0.47	2.97 ± 0.60	-1.61 ± 0.32 †
Plaque index	1.93 ± 0.69	1.86 ± 0.66	-0.07 ± 0.96	2 ± 0.71	1.35 ± 0.56	-0.64 ± 0.85 *
Gingival index	1.97 ± 0.54	1.80 ± 0.56	-0.16 ± 0.72	2.18 ± 0.62	1.03 ± 0.52	-1.15 ± 0.59 *
Marginal bone loss	2.04 ± 0.49	2.16 ± 0.53	+0.11 ± 0.27 #	2.12 ± 0.44	2.04 ± 0.50	-0.07 ± 0.41 #
GCF volume	1.41 ± 0.34	1.53 ± 0.42	0.15 ± 0.42	1.45 ± 0.42	0.88 ± 0.51	-0.57 ± 0.57 *

† $p<0.001$ * $p<0.01$ # $p<0.05$

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DISCUSSION

Although lasers have been used in dentistry for many years, systematic reviews of the literature report inadequate evidence to support their application in treatment of periodontal disease. In the series of clinical studies on which this thesis is based, the subjects comprised patients with moderately severe periodontitis, who underwent conventional treatment by scaling and root planing. The split-mouth studies then evaluated the potential of adjunctive application of therapeutic or surgical lasers to improve the short and long-term treatment outcomes. Clinical, microbiological and immunological parameters were recorded.

In the four studies undertaken, the first two using multiple applications of therapeutic lasers and the third and fourth using a single application of the Nd:YAG (surgical) laser, the overall results confirmed the beneficial effect of laser irradiation of the tissues after scaling and root planing. Sites which received laser irradiation exhibited improved clinical parameters and positive responses in terms of changes in inflammatory markers in gingival crevicular fluid. Moreover, in Study IV, the long-term outcome of a single application of the Nd:YAG laser also showed some gain in alveolar bone levels.

The initial study in the series confirmed that as a complement to SRP, LPT can reduce gingival inflammation. Adjunctive laser treatment resulted in significantly better clinical variables such as PPD, PI, GI, and GCF than SRP alone. The decrease in plaque index was also greater on the LPT side; this is in agreement with a study by Iwase *et al.* (1989). Significant decreases in GI and PPD have also been reported by Kiernicka *et al.* (2004). Ribeiro *et al* (2008) reported that LPT following SRP reduces gingival inflammation and MMP-8 expression, while histological examination showed a reduction in inflammatory cells. However, there are also some contradictory reports on the effectiveness of LPT. Rydén *et al* (1994) and Yilmaz *et al.* (2002) reported that LPT alone did not have an effect on the inflammatory response. Direct comparisons of studies are however, difficult, due to differences in wavelengths, energy output and irradiation mode. Further to that, Rydén *et al*. treated experimental gingivitis in healthy individuals. Such experiments, using healthy animals or humans have recently been

questioned. The genetically diabetic rat has, for instance, been a better model (al-Watban *et al.*2007).

Study I showed that MMP-8 decreased on the LPT side and increased on the SRP-only side, but the change did not reach significance (p=0.052). This in accordance with studies by Luza & Hubacek (1996) and Fujimaki *et al.* (2003).

The microbiota were unchanged in both studies I and II. This may be due in part to the timing of the sampling, after SRP, when the microbial load was already lowered. LPT in itself does not have any bactericidal effect, but stimulation of macrophages (el Sayed & Dyson 1996) could lead to phagocytosis and reduced bacterial load.

Ozawa *et al.* (1997) showed that LPT significantly inhibits the increase of plasminogen activator (PA) induced in human periodontal ligament cells in response to mechanical tension force. PA is capable of activating latent collagenase, the enzyme responsible for cleaving collagen fibers. LPT was also efficient in the inhibition of PGE_2 synthesis. In human gingival fibroblast culture, LPT significantly inhibited PGE_2 production stimulated by lipopolysaccharide (LPS) through a reduction of COX2 gene expression in a dose dependent manner. The decrease on PGE_2 levels in cultures of primary human periodontal ligament cells was also verified after cell mechanical stretching. Nomura *et al.* (2001) verified that LPT significantly inhibited LPS-stimulated IL-1ϐ production in human gingival fibroblasts cells, and that this inhibitory effect was dependent on irradiation time.

Safavi *et al.* (2008) evaluated the effect of LPT on gene expression of IL-1ϐ, interferon ɔ (IFN-ɔ) and growth factors (PDGF, TGF-ϐ and bFGF) to provide an overview of laser influence on their interactive role in the inflammatory process. The findings suggest an inhibitory effect of LPT on IL-1ϐ and IFN-ɔ production and a stimulatory effect on PDGF and TGF-ϐ. These changes may be explained the anti-inflammatory effects of laser and irradiation and its positive influence on wound healing.

Arany *et al.* (2007) in a study of the latent growth factor complex Transforming Growth Factor-ß (TGF- ß), a multifaceted cytokine reported that the latent form can be activated by LPT.

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The findings of the above studies, describing different pathways of inflammatory modulation, support the hypothesis explored in Study I that LPT can modulate the periodontal inflammatory process, especially through the reduction of PGE_2 release. In summary, LPT influences the expression of COX2 and IL-1ß, as well as MMP8, PDGF, TGF-ß, bFGF and plasminogen. However, the capacity of LPT to modulate inflammation does not seem to be confined to a single mechanism or to specific wavelength, fluence or power: the different parameters tested in various studies gave divergent results.

Study II demonstrated the importance of the coherence length of laser light. The clinical signs of inflammation were significantly decreased on the HeNe laser side (longer coherence length) compared to the diode laser side (short coherence length). Several studies comparing the biological effects of coherent and non-coherent light have reported that coherent light is superior (Hode 2005). In a study of regeneration of crushed optical nerves, the HeNe laser delayed the degenerative process, while non-coherent infrared light was ineffective (Rosner *et al.*1993). Similar conclusions have been drawn from other studies (Haina *et al.* 1973, Rochkind *et al.* 1989). It is claimed that coherent light is even more effective in deeper structures (Hode 2005). The cited studies compared coherent and non-coherent light, which has in fact a coherence length, albeit very minor. In Study II, two different coherent light sources of different coherence length were compared. The results confirmed the hypothesis that coherence length is an important determinant in laser phototherapy.

With respect to which wavelength best promotes cell proliferation, contradictory results are reported. However, other factors besides wavelength and the energy dose are important determinants of cell growth stimulation. Azevedo *et al.* (2006) tested two power densities (428.57 and 142.85 mW/cm^2) at the same energy density (2 J/cm^2) and showed that a lower power density caused higher stimulation. Moreover, the mode of exposure, pulsing or continuous, may also play a role in optimizing stimulation. The number of irradiation sessions and the treatment schedule will also influence the outcome. The power densities used in studies I and II are low and impractical from a clinical perspective. However, the design of the studies took into account

recommendations in the literature (Huang *et al.* 2010), that the use of low power densities over a longer treatment time would give an optimal outcome.

While pain is not a characteristic feature of chronic periodontitis, it is of major concern after SRP. LPT application can decrease the pain sensation. However, the applied dose must be considered closely. An approximate dose range of 2-6 J/cm^2 is considered optimal for wound healing and 6-10 J/cm^2 for hastening the inflammatory process. A shortening of the inflammatory process will in itself reduce the period of pain perception. A larger dose will cause an inhibition of neural transmission and a rapid decrease of pain (Chow *et al.* 2007). This dose is however, inhibitory for wound healing and will prolong the inflammatory process. In this context, it is important that the clinician understands the rationale underlying the laser application and is familiar with appropriate dose ranges.

Disruption of collagen fibres in the periodontal ligament is attributed mainly to the two collagenases MMP-1 and MMP-8. MMP-8 is released primarily from polymorphonuclear leukocytes (PMNL) and secreted predominantly into the GCF: thus MMP-8 levels in a GCF sample reflect the number of PMNL present and is an expression of the severity of inflammation (Tervahartiala *et al.* 2000). IL-1ϐ is a pro-inflammatory cytokine released mainly from monocytes/macrophages, and is present in the gingival tissues and GCF of patients with periodontal inflammation. Laser irradiation is associated with significantly greater reductions in MMP-8 and IL-1ϐ (Liu *et al.* 1999).

Thus laboratory analyses confirm the clinical signs of improved healing at these sites. The Liu study cited above compared the effects of SRP and SRP plus Nd:YAG laser on the laboratory markers of periodontal inflammation. The six to 12 week follow-up results showed a significant reduction in IL-1ϐ levels after treatment with SRP plus Nd:YAG laser compared to treatment by SRP alone. Similar results have been reported by (Choi *et al.* 2004 and Ge *et al.* 2008).

The present studies disclosed no differences between SRP and SRP + laser irradiation with respect to the cytokines IL- 1ϐ and IL-8, 6, and 4, and the total amount of elastase activity. Shimizu *et al.* (1995), in an *in vitro* study, reported that LPT affects the

production of cytokines. The discrepancy between *in vitro* and *in vivo* findings may be attributable to the fact that *in vitro* the actual energy density at the target would be considerably higher than in the clinical setting.

The relative effects of ultrasonic treatment, carbon-dioxide laser and Nd:YAG laser have been investigated in several studies. Nd:YAG laser (without water-cooling) and ultrasonic scaling resulted in significant improvements in clinical parameters (Israel *et al.* 1997; Spencer *et al.* 1996; Miyazaki *et al.* 2006).

In contrast to the results of Study III, Sjöström and Friskopp (2002) using a similar Nd:YAG laser, with water cooling, immediately following SRP, disclosed no additional benefit for laser irradiation at the four-month control. A reduced need for anaesthetics was the only obvious clinical advantage. The reason for the discrepant results is unclear; however, it might be attributable to differences in the study design: in the Sjöström study the laser was set to 7 W, in accordance with the manufacturerôs recommendations; whereas in Study III the setting was lower - 4 W.

A study by Lizarelli *et al.* (2006) showed that, within a limited range of power Nd:YAG laser is a safe tool for irradiation of primary teeth in a broad range of applications.

The laser fibre used in Study III was 600 µm in diameter and operated with a water cooling system. Compared to a 600 µm tip, the power density of the conventional 300 µm tip is four times higher, causing greater carbonization and tissue adherence, resulting in less control over the energy output at the tip. The 600 µm tip reduces the power density and so does the water spray (Gold and Vilardi 1994; Radvar *et al.*1996). In the present study, in order to overcome the loss of power at the fibre tip, the following settings were selected: 4 W, 80 mJ per pulse, 50 Hz, and a pulse width of 350 µs. A further advantage of the 600 µm tip is the reduced risk of fibre fracture. Results by Israel *et al.* (1997) showed that high energy, such as 9 W, can have negative effects on the root surface. However, no such damage is associated with laser treatment at 4 W and water coolant (Spencer 1996).

It is difficult to offer a comprehensive explanation for the greater improvement of periodontal status at the laser-irradiated sites. An important contributory factor may be

that laser application results in partial removal of the pocket epithelial lining. The reduction in PI and PPD at the test sites might be associated with the improvement in periodontal inflammation: because they experience less discomfort, patients may be able to brush more thoroughly and maintain good oral hygiene at these sites.

The bactericidal effect of Nd:YAG laser has been tested *in vitro* by Kranendonk *et al.* (2010). Suspensions of six different periodontal pathogens (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum* and *Parvimonas micra*) were prepared in small tubes and exposed to the Nd:YAG laser for five different intervals, using the following laser settings: Power 6 Watt, Pulse Repetition Rate 50 Hz, Pulse duration 250 ms. After exposure to the laser, aliquots of the suspensions were spread on blood agar plates for bacterial counting. After 5 s of laser exposure, there was a decrease in total colony forming units of all six selected micro-organisms. After laser irradiation for 15, 30 and 45 s, no viable bacterial cells remained.

In Study IV, sites irradiated with a single application of Nd:YAG laser as an adjunct to SRP showed a reduction in periodontal inflammation and bone loss compared to the control side. The improvement in clinical inflammation in terms of GI, was corroborated by the reduction of GCF volume on the test compared to the control side. Similar results have been reported previously (Wakao *et al.1989)* Laser irradiation has been proposed as an adjunct to conventional scaling and root planning in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

The present thesis is based on a series of four clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. Two different types of dental laser were investigated. Therapeutic lasers, which are claimed to stimulate cell regeneration and boost the immune system, were investigated in studies I and II: the general effect was investigated in Study I, while Study II compared the difference between gas and diode lasers in the same spectrum, in order to evaluate the importance of the length of coherence in biostimulation. In studies III and IV, the

surgical Nd:YAG laser, which is usually applied for sulcular debridement and pocket decontamination, was evaluated in a novel approach. The test procedure comprised one single application of the laser with water coolant after conventional scaling and root planing. In study III, the outcome was evaluated after 3 months and in Study IV the long term outcome was evaluated, at least one year post-treatment.

The split mouth design was used in all four studies. Study I showed a better clinical outcome on the laser treated side and some improvement in immunological parameters. The results of Study II support the hypothesis that a laser with a long length of coherence is superior to one of a shorter length, although both lasers had some positive clinical effect. In Study III a single application of the Nd:YAG laser as an adjunct to scaling and root planing improved the short-term outcome and Study IV confirmed that this improvement was sustained.

Besides reducing periodontal inflammation laser irradiation has been proposed as an adjunct to conventional scaling and root planning in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

Nd:YAG laser treatment also supports new connective tissue formation. A significant reduction in PPD with increased clinical attachment levels is associated with Nd:YAG laser therapy in patients with periodontitis (Yukna *et al.* 2007). This study demonstrated new cementum and connective-tissue formation, also reported subsequently by Romeo *et al.* (2009). Used at low energy, the Nd:YAG laser does not cause damage to the cementum or the dental pulp. An earlier *in vitro* study by Radvar *et al.* (1995) also showed that the Nd:YAG laser did not have a negative influence on cementum, suggesting the formation of new connective tissues around the periodontium.

New bone regeneration is a goal of periodontal therapy, but is seldom achieved. The receptor activator of the nuclear factor-kB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system is essential in bone turn over. An animal

study by Xu *et al.* (2009) investigated the effect of 650 nm irradiation on mRNA expression of receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) in rat calvarial cells. The authors concluded that the irradiation may directly promote osteoblast proliferation and differentiation, and indirectly inhibit osteoclast differentiation, by downregulating the RANKL:OPG mRNA ratio in osteoblasts. These observations support an earlier study by Kim *et al.* (2007).

Study IV showed minor bone loss on the SRP only side while the side treated with laser and SRP showed some bone gain. Similar results have been reported in a recent experimental study in rats (de Almeida *et al.* 2008). While more bone regeneration is reported in some clinical studies (Kim *et al.* 2010), in most such studies the selected subjects exhibited more severe periodontitis at baseline, with pockets >4 mm, whereas in the present series of studies the inclusion criteria stipulated that pocket depth should not exceed 4 mm. Another difference in study design concerned the number of laser applications: better bone regeneration was recorded in studies in which the subjects underwent several laser therapy sessions, while the present studies III and IV included only one session of Nd:YAG irradiation. While one such session may therefore not be optimal, it appears to have been effective.

There are obvious weaknesses in Study IV, such as the small number of participants, the relatively long unsupervised period and varying observation times, and the outcome of only minor differences in alveolar bone height between the groups. A difference in bone level of 0.18 mm is not clinically relevant. However, it is statistically significant and shows that one application of Nd:YAG laser can have a long-term beneficial effect on alveolar levels.

In conclusion, the results of these studies confirm the potential role of laser irradiation as a non-invasive adjunctive to scaling and root planing in the treatment of periodontitis.

Key words: Low level laser, Nd:YAG laser, protease activity, coherence length, periodontal inflammation, cytokines, scaling and root planing.

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OVERALL CONCLUSIONS

Study I showed that compared to SRP alone, additional treatment with LPT significantly reduced periodontal gingival inflammation.

Study II showed that in laser phototherapy, a gas laser was more effective than a diode laser in reducing gingival inflammation.

Study III showed that compared to SRP alone, an additional single application of a water cooled Nd:YAG laser significantly improved clinical signs associated with periodontal inflammation.

Study IV showed a long-term positive effect of a single application of Nd:YAG laser in combination with SRP.

FUTURE PERSPECTIVES

A review of the literature confirms that the outcome of laser applications in dentistry is heavily dependent on the parameters selected. With sufficient knowledge, lasers can be used for multiple applications and could be a substantial addition to the armamentarium of the periodontist as well as the general dentist. But considering the great variability of the available parameters, more research is necessary to identify therapeutic windows for each indication and for each wavelength. Only then will dental lasers be more readily accepted and sold in greater numbers, at prices that most dentists will consider affordable. Researchers involved in this field have an obligation to be active in education activities to ensure that dental lasers are applied in an evidence-based, professional way. Future studies should preferably be multi-centre studies, where all centres have identical equipment and methods. The present literature is difficult to interpret due to lack of uniformity in selected parameters.

The reduction of the pocket microflora is an interesting topic. It is obvious that Nd:YAG laser can reduce the bacterial burden, but to date there are few published studies in this field.

In contrast to SRP, Nd:YAG laser can remove the pocket epithelial lining. The practical importance of this property needs further verification. A negative outcome is not necessarily attributable to lack of effect of the laser, but may be due to unsuitable power settings, pulse repetition rates, total energy, treatment technique and fibre size. The present series of studies highlights the importance of the fibre size. Further studies are warranted to elucidate the influence of different fibre sizes on the clinical outcome.

The two Nd:YAG studies in this thesis have deliberately used a closed pocket mode, in order to be able to compare the additional effect of the Nd:YAG laser after SRP. However, a more surgical approach is also possible, where the pocket is opened during the removal of the pocket epithelial lining, offering the operator a better view of the pocket, allowing improved inspection of remaining debris. This technique also needs to be investigated in future studies.

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As therapeutic lasers and the Nd:YAG laser were investigated in this thesis and both exhibited beneficial effects, a combined study would be of interest. After reducing the bacterial load and the epithelial lining, a number of subsequent applications of LPT could further improve healing by stimulating periodontal cells such as precursors to osteoblasts. The adjuvant effect of LPT in traditional periodontal treatment modalities such as GTR and organic and/or inorganic bone substitutes should also be highlighted. The anti-inflammatory effect of LPT also needs to be better understood.

There are other lasers on the market such as diodes and Er:YAG. The application of these in periodontology also warrants investigation.

Although the use of different lasers in periodontology has not been extensively investigated, the literature suggests many potential advantages. Future research should focus on establishing such an evidence-based treatment modality.

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The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation

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Abstract

Objectives: The aim of this split-mouth, double-blind controlled clinical trial was to study the effects of irradiation with low-level lasers as an adjunctive treatment of inflamed gingival tissue.

Materials and Methods: Seventeen patients with moderate periodontitis were included. After clinical examination, all teeth were scaled and root planed (SRP). One week after SRP, we took samples of gingival crevicular fluid (GCF) and subgingival plaque. The laser therapy was started 1 week later and continued once a week for 6 weeks. One side of the upper jaw was treated with active laser and the other with a placebo. The test side was treated with two low-level lasers having wavelengths of 635 and 830 nm. The patients then underwent another clinical examination with sampling of GCF and plaque. The GCF samples were analysed for elastase activity, interleukin-1β (IL-1β) and metalloproteinase-8 (MMP-8). We examined the subgingival plaque for 12 bacteria using DNA probes.

Results: The clinical variables i.e. probing pocket depth, plaque and gingival indices were reduced more on the laser side than on the placebo one ($p < 0.01$). The decrease in GCF volume was also greater on the laser side, 0, 12 μl, than on the placebo side, 0.05 μl ($p = 0.01$). The total amount of MMP-8 increased on the placebo side but was slightly lower on the laser side ($p = 0.052$). Elastase activity, IL-1β concentration and the microbiological analyses showed no significant differences between the laser and placebo sides.

Conclusion: Additional treatment with low-level lasers reduced periodontal gingival inflammation.

Key words: low-level laser; periodontal inflammation; protease activity; therapeutic laser

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Lasers have been used in dentistry since the beginning of the 1980s. In oral surgery, the carbon dioxide laser (CO_2) has become an accepted method for the removal of superficial layers without damaging underlying tissues and for its excellent coagulating effects. More recently the Er:YAG laser was introduced because of its versatile properties e.g., the ablation of hard and soft tissues. Several lasers have been used to sterilize root canals and periodontal pockets. The Nd:YAG laser is useful for debridement of calculus and reduction of endodontic microbes inter alia (Gutknecht et al. 1996). While surgical lasers such as these are routinely used in modern dentistry, low-level lasers (also known as therapeutic lasers) have been utilized less frequently. Low-level lasers do not cut or ablate but are based on photobiological processes (Karu 2003). Unlike the powerful surgical lasers that require >1 W, these lasers function in the milliwatt range with wavelengths usually in the red and near-infrared spectrum and can be used to change intra-cellular photoreceptors, e.g. endogenous porphyrins, flavoproteins and cytochrome c-oxidase in the respiratory chain (Karu 2003). The absorption leads to a cascade of photobiological events, which could have advantageous effects on periodontal healing. For example an increased cell metabolism and collagen synthesis have been shown in fibro-

blasts, and an increased activity of leukocytes and release of growth factors have also been suggested. Cells in a reduced state respond best to laser irradiation (Yamamoto et al. 1996, Karu 2003). Low-level lasers have been used for more than 30 years and no adverse effects have been reported. The US Food and Drug Administration lists these lasers as *non-significant risk Class III medical devices* and several of these have been approved. No noticeable increase in temperature occurs and patients readily accept the therapy.

In this study we used two lasers, Indium–Gallium–Aluminium–Phosphide (InGaAlP, 635 nm) and Gallium–Aluminium–Arsenide (GaAlAs, 820 nm). The InGaAlP laser was chosen because this wavelength seems to have good effects on the mucosa and gingiva (Loevschall & Arneholt-Bindslev 1994) and because of the 10 year's experience of one of the authors (T. Q.) concerning this wavelength for treatment of gingivitis and periodontitis. The GaAlAs laser was added to improve the penetration of light into the periodontal and bony areas (Saito & Shimizu 1997).

The positive effects of therapeutic lasers in dentistry have been reported for such diverse conditions as mucositis (Bensadoun et al. 1999), paresthesia (Khullar et al. 1996), HSV-1 (Schindl & Neumann 1999), temporomandibular disorders (Kulekcioglu et al. 2003), dentine hypersensitivity (Kimura et al. 2000) and osseointegration (Dörtubak et al. 2002). In vitro studies have primarily concentrated on the fibroblast. Several authors report stimulation of gingival fibroblast proliferation after the use of low-level laser (Yu et al. 1996, Almeida-Lopes et al. 2001) and have shown that the stimulated fibroblasts are better organized, in parallel bundles (Almeida-Lopes et al. 2001).

No study has been done on the value of low-level laser irradiation as an adjunct to conventional scaling and root planing (SRP). We therefore investigate the clinical use of a combination of two therapeutic lasers on gingival inflammation.

Material and Methods

Participants and study design

Seventeen patients (10 women), mean age 53 (35–70) years, with moderate chronic periodontitis were selected for this study. To be included the patients had to be 35 years of age or older, have no ongoing general disease and be on no medication. Those who had taken an antibiotic during the last 4 weeks, had teeth with a mobility rate of II, III or pockets deeper than 7 mm in the areas studied were excluded. As it turned out, none of the participants had taken any antibiotics during the last 6 month. Patients with an acute condition in the mouth or partial dentures in the upper jaw were also excluded. Five patients were smokers. Some of the participants had had periodontal treatment earlier but none had received laser treatment before.

Initially, all participants received basic periodontal treatment including scaling, root planing and oral hygiene instructions. Baseline measurements of the probing pocket depth (Perio Wise, Premier, Canada), gingival index (GI, Silness & Löe 1967) and plaque index (PI, Löe 1964) were recorded before the SRP. Gingival cervicular fluid (GCF) samples, for analyses of elastase, IL-1β and metalloproteinase-8 (MMP-8), and subgingival plaque samples were taken 1 week after SRP. One of the authors (T. Q.) did both baseline and follow-up examinations as well as the SRP on all patients. After another week a laser therapist started the low-level laser therapy.

The test or control areas comprised teeth 13, 14, 15, 16, 17 and 23, 24, 25, 26, 27. One side was treated with the active laser and the other with the placebo laser once a week for 6 weeks. One week after the last laser irradiation, the clinical examination and GCF/plaque sampling were done in the same way as at baseline. The laser therapist randomly allocated the quadrants for active laser or placebo. The clinical examiner did not know which side had been treated with active laser until the completion of the study. This study was approved by the Ethics Committee of Huddinge Hospital, Sweden.

Laser treatment

We employed a handheld battery-operated Combilaser (Lasotronic AG, Baar, Switzerland), which has two wavelengths that can be used together or separately. In this study the wavelengths were utilized separately. Two identical units were used. In the placebo unit the laser diode was replaced by a very low-powered red LED diode. The laser wavelengths were 635 (visible) and 830 (invisible) nm and the outputs, controlled daily with an analogue power metre (Lasotronic AG, Baar, Switzerland), 10 and 70 mW. Since all battery-powered tools lose power as the batteries deteriorate, the batteries were changed after each day of use. We treated (1) the buccal papillae with 635 nm laser for 90 s (0.9 J) and (2) 6 mm more apically with 830 nm for 25 s (1.75 J), from the buccal and lingual sides.

The energy densities were 4.5 and 8.75 J/cm^2 and the power densities 50 and 350 mW/cm^2. The treatment was given during slight contact with the tissue.

Samples

In all patients, two GCF samples were taken from each side of the upper jaw after removal of supragingival plaque from the sites to be sampled. These had been isolated with cotton rolls and gently dried with an air syringe before sampling. GCF was collected with prefabricated paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA), which were inserted into the pockets until resistance was felt and kept there for 30 s.

Blood-contaminated samples were discarded. We measured GCF volume with a calibrated Periotron™ 8000 meter (Oraflow Inc.). The two samples from each side of the upper jaw were pooled together and diluted in phosphate buffer saline (PBS) up to 1 ml. After elution for 15 min., the strips were removed and the samples frozen at -20°C pending analysis. Subgingival plaque was sampled from the same sites with sterile paper points (size30), which were inserted for 30 s. The paper points from each side were then pooled together in sterile transport vials and sent to a laboratory for bacterial DNA-probe analysis.

Laboratory analyses

IL1-β was measured as described elsewhere (Figueredo et al. 1999). Briefly, a monoclonal antibody to IL1-β (MAB 601, R&D Systems, Minneapolis, MN, USA), diluted 125 times in carbonate buffer, was coated onto microtitre plates (Nunc Maxisorb, Nanc a/s, Roskilde, Denmark) overnight at $+4^{\circ}$C. These were washed once, with PBS+0.05% polyoxyethylenesorbitan monolaurate (Tween® 20, Sigma Chemical, St. Louis, MO, USA), and blocked with 1% HSA for 1 h at room temperature. After four washings, a standard curve (2 –200 pg/ml) and undiluted samples (100 μl) were added to the plates. They were incu-

bated at +37°C while shaking for 45 min. and then washed four times. The detection antibody (BAF 201, R&D Systems), a biotinylated polyclonal goat antibody diluted 250 times, was incubated as described above. After washing, the horseradish peroxidase conjugated streptavidin, diluted 200 times in PBS+0.1% HSA, was added to the plates and incubated in the same way as the detection antibody. The plates were washed again and the undiluted substrate (TMB, Sigma Chemical) added. The reaction was stopped with 1 M H_2SO_4 after 15 min. and the absorbency read at 450 nm in a spectrophotometer (Millenia Kinetic Analyser, Diagnostic Product Corporation, Los Angeles, CA, USA).

The total elastase activity was measured with a chromogenic substrate specific for granulocyte elastase. One hundred microlitres of undiluted sample was mixed with 65 μl of substrate S-2484 (L-pyroglutamyl-L-propyl-L-valine-*p*-nitraniline, mw 445.5 Da, Heamochrome Diagnostica, Mölndal, Sweden) on a 96-well microtitre plate (Nunc Maxisorb, Nunc a/s,). The mixture was shaken for 5 min. and the absorbency at 405 nm was read in a spectrophotometer. After 2 h of incubation at 37°C, the absorbency was read for the second time. The total elastase activity is expressed in mAbs (milliabsorbances).

MMP-8 was analysed with a commercial kit (Quantikine®, R&D Systems Inc.) in accordance with the manufacturer's instructions. Briefly, a monoclonal antibody specific for MMP-8 had been pre-coated onto a microplate. Samples diluted 10 times and a standard curve were pipetted into the wells and incubated at room temperature for 2 h. The plates were then washed and a monoclonal antibody against MMP-8 conjugated to horseradish peroxidase was added and incubated again as before. After another washing procedure, the substrate solution was added and the reaction stopped after 15 min. with a stop solution. The absorbency at 450 nm was read within 20 min. in a spectrophotometer.

The subgingival microbiota was analysed using a checkerboard DNA–DNA hybridization method. The 12 microorganisms tested with the DNA probe in the subgingival samples were: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythensis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Treponema denticola, Peptostreptococcus micros, Campylobacter rectus, Eikeinella corrodens, Selenomonas noxia* and *Streptococcus intermedius*. We used standard procedures for the checkerboard DNA–DNA hybridization method (Papapanou et al. 1997) and recorded the frequencies of positive sites and of sites with $\geqslant 10^6$ of these bacteria.

Statistical analysis

The unit of analysis was the subject. The significance of the differences in treatment effect between placebo and laser was calculated with the Student paired *t*-test or the Wilcoxon-signed rank test. The frequencies of positive subjects and of subjects with $\geqslant 10^6$ of the analysed bacteria were calculated with Fisher's exact test.

Results

Baseline probing depth was 4.7 (0.7) mm on the laser side and 4.7 (0.6) mm on the placebo side. After treatment the probing depth was 3.8 (0.6) mm on the laser side and 4.5 (0.6) mm on the placebo side. The probing depth reduction was significantly larger on the laser side (Table 2). Baseline and follow-up values of gingival and plaque are shown in Table 1. Both gingival and plaque index were reduced more on the laser-treated side ($P < 0.001$).

The changes in the laboratory variables after laser or placebo treatments are shown in Table 3. After treatment, the GCF volume was reduced by 0.14 μl on the side given additional treatment with laser, while the volume was reduced by 0.04 μl on the placebo-treated side.

We found a tendency to a reduction in MMP-8 on the laser-treated side ($p = 0.052$). On the laser side, the mean amount of MMP-8 fell by 100 pg, but increased by 274 pg on the placebo side. No significant differences were observed in elastase activity and the amount of IL-1β (Table 3).

As regards the subgingival microbiota, no differences were detected between laser and placebo sides in the frequencies of positive subjects or of subjects with $\geqslant 10^6$ of the 12 bacteria analysed (Table 4).

Discussion

In this study we showed that additional treatment with low-level laser reduced the gingival inflammation after non-surgical treatment. Both gingival index and probing pocket depth declined more on the side given such treatment. Another marker of inflammation, the GCF volume (Oliver et al. 1969), also fell more on the laser side. One explanation may be that laser irradiation reduces prostaglandin PGE_2 (Sakurai et al. 2000). The stimulation of cellular ATP (Karu 2003) could be another contributory factor.

The decrease in plaque index was also greater on the laser side, which agrees with an earlier animal study

Table 1. Gingival and plaque index at baseline and after scaling, root planing and adjuctive treatment with active or placebo laser

	Gingival index (median (range), mean (SD))		Plaque index (median (range), mean (SD))	
	baseline	follow-up	baseline	follow-up
Placebo ($n = 17$)	2 (1–3), 2.2 (0.5)	2 (0–3), 1.7 (0.7)	1 (0–3), 1.4 (0.6)	1 (0–2), 1.1 (0.7)
Laser ($n = 17$)	2 (1–3), 2.3 (0.6)	1 (0–2), 0.9 (0.8)	2 (0–2), 1.6 (0.6)	1 (0–2), 1.0 (0.6)

Table 2. Mean values (SD) of probing pocket depth and GCF volume before and after treatment with active laser or placebo

	Probing pocket depth (mm)			GCF volume (μl)		
	baseline	follow-up	change	baseline	follow-up	change
Placebo ($n = 17$)	4.7 (0.6)	4.5 (0.6)	0.1 (0.3)	0.41 (0.15)	0.41 (0.15)	−0.05 (0.13)
Laser ($n = 17$)	4.7 (0.7)	3.8 (0.6)	0.9 (0.4)	0.44 (0.15)	0.29 (0.13)	−0.12 (0.11)
p^*	0.84	<0.001	<0.001	0.56	0.41	0.02

*p values calculated with Student's paired *t*-test. GCF, gingival crevicular fluid.

Table 3. Mean values (SD) of elastase activity, total amounts of IL-1β and MMP-8 between samples taken before and after treatment with active laser or placebo

	Elastase activity (mAbs)			IL-1β (pg)			MMP-8 (pg)		
	baseline	follow-up	change	baseline	follow-up	change	baseline	follow-up	change
Placebo ($n = 17$)	45 (3–324)	34 (2–611)	9 (− 576 to 252)	20.7 (5.1–49.7)	17.2 (1.3–71.3)	1.7 (57.9 to 24.7)	415 (0–1040)	465 (210–2940)	90 (2180 to 585)
Laser ($n = 17$)	17 (3–337)	32 (2–269)	32 (23 to 160)	21.0 (5.6–123.3)	21.0 (6.1–65.4)	0.8 (24.4 to 82.8)	500 (160–1600)	425 (0–1015)	70 (510 to 1145)
p^*	0.80	1.0	0.15	0.80	0.80	0.45	0.15	0.15	0.052

*p-values calculated with Wilcoxon's signed-rank test. MMP-8, metalloproteinase-8; mAbs, milliabsorbances.

Table 4. Percentage of positive samples (A) and of samples with $\geqslant 10^6$ bacteria (B) of indicated species, before and after treatment with laser or placebo. $N = 17$ subjects.

	Laser				Placebo			
	before		after		before		after	
	A	B	A	B	A	B	A	B
P. gingivalis	17.6	0	11.8	0	17.6	0	11.8	0
P. intermedia	29.4	11.8	29.4	5.9	29.4	5.9	35.3	0
P. nigrescens	41.2	5.9	35.3	0	35.3	5.9	35.3	0
T. forsythensis	47.0	0	41.2	0	41.2	0	35.3	0
A. actinomycetemcomitans	11.8	0	5.9	0	11.8	0	5.9	0
F, nucleatum	17.6	0	23.5	0	29.4	0	41.2	0
T. denticola	52.9	0	64.7	0	64.7	0	35.3	0
P. micros	64.7	0	64.7	0	82.4	0	76.5	0
C. rectus	17.6	0	5.9	0	11.8	0	0	0
E. corrodens	23.5	0	23.5	0	23.5	0	17.6	0
S. noxia	5.9	0	5.9	0	11.8	0	11.8	0
S. intermedius	64.7	0	64.7	0	70.6	0	76.4	0

There were no significant differences between the laser and placebo sides.

(Iwase et al. 1989). It is uncertain whether this is because of a reduction in the degree of inflammation or the laser irradiation per se. However, the microbial analyses showed no differences between the laser and placebo sides in prevalence of subjects with positive findings or of those with $\geqslant 10^6$ of each bacteria. A previous in vitro study of the effect of laser irradiation on microorganisms has found that the growth of *Streptococcus mutans* is stimulated by laser (Kim et al. 1992). However, in another clinical and histological study by the same authors (Kim & Lee 1987) the number of motiles and spirochetes declined while that of the non-motiles increased. This finding was not confirmed by our study. Some authors have reported that a combination of low-level laser light with various dyes, such as toluidine blue O (TBO), significantly reduces the number of subgingival microorganisms. In such cases the laser activates the bactericidal effects of the dye and does not act directly on the microorganisms (Wilson et al. 1995).

We found that additional irradiation with low-level laser was better than scaling and root planing alone. Its effect was greatest on the gingival index and probing pocket depth. The beneficial effect on gingival inflammation was also shown by the marked decrease in the volume of GCF. In a study by Yilmaz et al. (2002), laser alone did not affect the inflammatory response more than instructions about oral hygiene. Mechanical subgingival debridement was necessary. However, the outcome in the group receiving subgingival debridement and laser was only slightly better than in the group given subgingival debridement alone.

Our analyses of GCF showed a slight decrease in the amounts of MMP-8 on the laser side and an increase on the placebo side. MMP-8 is stored in the secretory granula of neutrophilic granulocytes and released from the cells to the inflammatory lesion during migration (Bentwood & Henson 1980). It can therefore be regarded as a surrogate marker of the number of neutrophils in the area and as a marker of the severity of inflammation. In vitro irradiation of peripheral neutrophils affects neutrophil functions such as the generation of reactive oxygen species and phagocytosis (Luza & Hubacek 1996, Fujimaki et al. 2003).

In the present study, no effect was found on neutrophil phagocytosis, measured as elastase release, i.e. degranulation of primary granula.

Some data suggest that laser irradiation affects the production of cytokines (Shimizu et al. 1995), but our study did not confirm the occurrence of inhibition of IL1-β, which has been reported by others (Shimizu et al. 1995). This may be because the previously cited studies were done in vitro and the actual energy density at the target was therefore considerably higher.

It is not always possible to select the optimal laser and treatment parameters for laser therapy because of the lack of adequate studies. The parameters used in this study seem to have been within the ''therapeutic window'' of dosage but not necessarily optimal. Many studies have failed to find this window,

especially in studies performed in the 1980s and early 1990s (Tuner & Hode 1998). Many authors used doses in the range of 0.001–0.01 J/cm^2 (Masse et al. 1993) although it had been suggested by Mester et al. as early as 1971 that doses of about 1–2 J/cm^2 are necessary to heal wounds.

Some of the effects of laser therapy may be because of an increase in the microcirculation in the irradiated area (Schaffer et al. 2000). In the study of gingival microcirculation using healthy volunteers with experimental gingivitis, no effects were seen (Rydén et al. 1994), but other authors have shown that low-level laser affected the microcirculation in mildly inflamed gingiva, but not in uninflamed or severely inflamed gingiva (Kozlov et al. 1995). On the other hand, when the microcirculation in the masseter muscle was studied (Tullberg et al. 2003), no increase in microcirculation occurred in tender areas, but a significant increase was noted in similar locations in healthy volunteers.

A suggested aspect of laser therapy is the so-called systemic effect, which implies that if a pathological condition on one side of the body is irradiated, a small but noticeable effect would be obtained on a similar condition on the other side of the body (Rochkind et al. 1989). The design of our present study does not allow us to investigate this effect.

In conclusion, the additional treatment with therapeutic laser reduced the periodontal inflammation, as assessed by the gingival index, probing pocket depth, GCF volume and MMP-8 levels.

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ORIGINAL ARTICLE

The importance of coherence length in laser phototherapy of gingival inflammation—a pilot study

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Abstract The aim of this study was to investigate if coherence length is of importance in laser phototherapy. Twenty patients with moderate periodontitis were selected. After oral hygiene instructions, scaling and root planing (SRP), one side of the upper jaw was randomly selected for HeNe (632.8 nm, 3 mW) or InGaAlP (650 nm, 3 mW) laser irradiation. One week after SRP, the following parameters were measured: pocket depth, gingival index, plaque index, gingival crevicular fluid volume, matrix metalloproteinase (MMP-8), interleukin (IL-8) and subgingival microflora. The irradiation (180 s per point, energy 0.54 J) was then performed once a week for 6 weeks. At the follow up examination, all clinical parameters had improved significantly in both groups. A more pronounced decrease of clinical inflammation was observed after HeNe treatment. MMP-8 levels were considerably reduced on the HeNe side, while there was no difference for IL-8 or microflora. Coherence length appears to be an important factor in laser phototherapy.

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Introduction

Gingivitis and periodontitis are very common diseases among adults. In a Swedish population, approximately 90% have gingivitis, 60% show signs of periodontitis, while 7% have severe periodontitis [1]. Gingivitis is described as a reversible inflammation of the gums. Clinical signs include redness, swelling, and in severe cases, bleeding. Periodontitis is a chronic inflammation that degrades the tissues attaching the tooth to the jaw bone. Eventually, periodontitis can result in tooth loss and edentulousness. Both these conditions are induced by microorganisms colonising the gingival sulcus. Conventional treatment consists of mechanical removal of the microorganisms by scaling, root planing (SRP) and polishing, in combination with the patient's own oral hygiene measures to remove the bacterial plaque. However, this treatment is not always sufficient.

Treatment with high-output lasers such as Nd:YAG, Er:YAG, diodes and CO_2 have been used in periodontal practise for many years. The wavelength and output of each of these lasers differ, and attention has to be paid to the advantages and limitations. Several studies have, however, reported a successful outcome of laser irradiation as an adjuvant therapy to conventional treatment [2–5], but the usage is not quite uncontroversial [6].

Treatment with therapeutic lasers or "low-level lasers" is less common, and little has been published concerning periodontal applications. Therapeutic lasers do not cut or ablate but are based on photobiological processes [7]. Unlike the powerful surgical lasers, these latter lasers

perform in the milliwatt range, with wavelengths usually in the red and near-infrared spectrum. Among the suggested photoreceptors are endogenous porphyrins, flavoproteins and cytochrome *c*-oxidase in the respiratory chain [7]. The absorption of the light stimulates a cascade of photobiological events. Cells in a reduced state respond best to laser irradiation [7, 8].

The positive effects of therapeutic lasers in dentistry are reported for a number of conditions such as mucositis [9], paresthesia [10, 11], herpes simplex type 1, [12], temporomandibular disorders [13–15], dentine hypersensitivity [16] and osseointegration [17–19]. The most common in vitro object for study is the fibroblast. Several authors have reported a stimulation of gingival fibroblast proliferation after laser irradiation [20–22]. Ozawa et al. [23] reported a reduction in stretching-induced plasminogen activator activity in human periodontal ligament cells, which suggested that laser irradiation may reduce collagen breakdown associated with traumatic occlusion. The clinical effect of therapeutic laser after gingivectomy is reported by Amorim et al. [24]. Kawamura et al. [25] reported that GaAlAs laser irradiation could reduce epithelial down growth into the pocket after flap operations.

Previously, we have [26] demonstrated a positive effect on gingival inflammation, using a combination of Helium–Neon (HeNe) and GaAlAs lasers. The aim of the present study was to further examine the possible mechanisms behind the obtained results. One previously unattended parameter in laser phototherapy research is the length of coherence of the laser light. It was hypothesised that the longer coherence length of the HeNe laser would have a more pronounced biological effect than a diode laser of the same wavelength and power.

The importance of the coherency has not been studied extensively, and it has even sometimes incorrectly been claimed that coherency is lost when light is diffusely scattered in the tissue, implying that coherency is not necessary at all. However, the fact that coherency is important in the treatment of bulk tissue is documented in some 20 studies comparing coherent and noncoherent light [27]. There is up until now no in vivo study comparing coherent and noncoherent light, suggesting that the effects are equal.

Coherency, in general, is the property of wave-like states that enables them to exhibit interference. It is also the parameter that quantifies the quality of the interference, also known as the degree of coherence. It was originally introduced in connection with Young's double-slit experiment in optics but is now used in any field that involves waves, such as acoustics. The degree of coherence is equal to the interference visibility, a measure of how perfectly the waves can cancel due to destructive interference.

Coherency of light is a complicated phenomenon. Photons in a coherent laser beam follow a certain statistical distribution regarding the temporal distance from one to the other. This distribution is the Poisson distribution, while non-laser light, for example, light of a thermal light source, obeys the very different Bose–Einstein distribution. Furthermore, the wave model of light is a model to describe the propagation of light in transparent media. In contrast, the photon is introduced by a completely different model, the quantum model of light, which is used to describe the interaction of light with matter. Two types of coherency are at hand, temporal coherency, where phase synchronization is valid for a certain time, and spatial coherency, meaning that light waves show coherency when they are emitted from different locations of an extended light source. In bulk tissue, laser speckles are formed through interference, and their contrast depends on the degree of spatial coherence of the light, which in turn, depends on the bandwidth of the laser light.

All diode lasers do not have the same bandwidth and coherency. The laser diodes used in therapeutic lasers are not very sophisticated; they are usually of multimode type, and an external resonator is never used. The HeNe laser in this study was of nonpolarized type and with a bandwidth of about 0.02 nm. The free-running bandwidth of the laser diode was about 2.0 nm. As the length of coherency can be estimated as $\lambda^2/\Delta\lambda$, it would mean that the length of coherency differs by a factor 100. However, a certain reduction of the coherence length takes place in the transmission through the fibre. But compared to the reduction of the coherency due to the scattering in tissue, this factor is probably negligible. The bandwidth of the different lights, respectively, in tissue is unchanged.

The effect of laser irradiation on gingival inflammation has been reported in a study by Qadri et al. [26]. In a split mouth study, the effect of laser light on gingival inflammation was investigated. The laser parameters used indicated that all clinical variables improved as well as some of the laboratory variables. In the study, one side of the mouth was treated with laser, and the opposite side was used as control. In spite of the possibility of systemic effects, the clinical and laboratory findings suggested that the model could be a base for studying the importance of the coherence length. The objective of the present pilot study was then to study whether or not the degree of coherence is of any importance and not only the coherence itself.

Materials and methods

Study population

After informed consent, 20 patients were selected for the study; 9 male and 11 female patients. The mean age was 51 years (SD), with a minimum age of 35 years. The peri-

odontal condition was assessed as light to moderate chronic periodontitis according to the 1999 classification [28]. No pockets should be >7 mm in the experimental area. No acute inflammatory processes, such as marginal abscesses or periapical lesions, were allowed. Patients with partial dentures in the upper jaw were not included. Three patients were smokers. Patients were not to take antibiotics of any kind during the 4 weeks before the beginning of the study.

Experimental design

The clinical parameters registered included probing pocket depth (PPD, Perio Wise, Premier, Canada) plaque index (PI) [29] and gingival index (GI) [30]. A dental surgeon recorded the clinical data, did the SRP and informed the patients how to perform their home care. Gingival crevicular fluid (GCF) was collected with paper strips (Periopaper, Oraflow, Plainview, NY, USA). The strip was inserted into the pockets/crevices until resistance was felt and kept there for 30 s. Blood-contaminated samples were discarded. The GCF volume was measured with a calibrated Periotron 8000 meter (Oraflow, Plainview, NY, USA). Each sample was eluted in phosphate-buffered saline (PBS) for 15 min; then, the strips were removed and the samples frozen at −20°C until analysis. The collected GCF samples were analysed for matrix metalloproteinase (MMP-8) and interleukin (IL-8). Furthermore, the presence of periopathogens was assessed through DNA analyses; all in all, 80 samples before and after the laser phototherapy sessions. The baseline procedures were performed not later than 1 week before laser phototherapy. The study had been approved by the Regional Ethical Review Board in Stockholm.

Laser irradiation

One side of the upper jaw in each patient was randomly assigned for HeNe irradiation and the contra lateral side for diode laser irradiation. Randomisation and laser irradiation were performed by a dental hygienist. The lasers used were a 3-mW HeNe laser (632.8 nm) from Irradia AB, Stockholm, Sweden and a Pocket Therapy diode laser (nominally 635 nm) from Lasotronic AG, Zug, Switzerland, equally of a nominal power of 3 mW. Both lasers had the same size of the aperture (2 mm in diameter), allowing for equal power densities of approximately 100 mW/cm^2. The wavelength of the diode laser was measured in a spectrometer and found to be 650 nm instead of the reported 635 nm. Laser diodes in the 630-nm range require cooling and are generally not found on the therapeutic market place. The possible implications of this are found in the discussion.

The output of the HeNe laser was measured in 7 min and found to be practically constant. In 7 min of radiation, the power of the diode laser first increased up to 3.2 mW (in 2 min) and then slowly fell to 2.9 mW. During the actual therapy, the laser was shut off for 10 s between each point of irradiation. The laser outputs were controlled weekly using analogue power meters provided by the manufacturers. The HeNe laser light was delivered through an optical fibre (flexible fibre bundle with 2 mm circular aperture), and the output power was measured at the fibre aperture. The length of coherence is reduced during the transmission in the fibre but is still much longer than that of the diode laser. The diode laser light was conducted through a stiff glass rod, the aperture of which was circular with a diameter of 2 mm.

Laser phototherapy started 1 week after baseline, with one session every week for 6 weeks. The laser treatment was performed by holding the laser probe in light contact with the tissue for 180 s per point, providing an energy of 0.54 joules (J). Each buccal papilla of teeth 13, 14, 15, 16, 17, 23, 24, 25, 26, 27 and the lingual papillae of 16 and 26 were irradiated. Total energy per quadrant was, hence, 3.24 J. Final clinical recordings and GCF sampling were done 1 week after the last laser session.

Laboratory analyses

MMP-8 and IL-8 were analysed with commercial kits (Quantikine, R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. Samples, diluted ten times for MMP-8 or undiluted for IL-8, and standard curves were pipetted into the wells of a microtitre plate, precoated with a monoclonal antibody against MMP-8 or IL-8. The plates were incubated at room temperature for 2 h. The plates were washed, and a horseradish peroxidase-conjugated polyclonal antibody against MMP-8 or IL-8 was added and incubated as before. After another washing procedure, the substrate solution was added, and the reaction stopped after 15 min with a stop solution. The absorbency at 450 nm was read within 20 min in a spectrophotometer. The amount of MMP-8 was expressed in nanograms (ng) and the amount of IL-8 in picograms (pg) per site.

The subgingival microbiota was analysed using a checkerboard DNA–DNA hybridisation method. Twelve microorganisms were tested with the DNA probe in the subgingival samples and included: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythensis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikeinella corrodens*, *Selenomonas noxia* and *Streptococcus intermedius*. Standard procedures for the checkerboard DNA–DNA hybridisation method were used [31] and the frequencies of positive sites recorded.

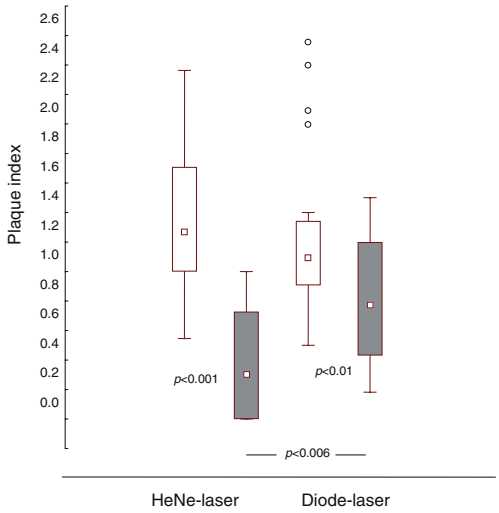

Fig. 1 Plaque index before and after laser treatment. *Filled boxes* indicate the results after treatment. The *box plots* show median, 75 and 90% range and outliers. Indicated *p*-values calculated with Wilcoxon signed rank test

Statistical analyses

Neither clinical nor laboratory variables were normally distributed. Thus, the significances of the differences in treatment effect between the two lasers were calculated with Wilcoxon signed rank test.

Table 1 Summary of clinical changes after treatment with diode laser or HeNe laser

Parameter	Change with diode laser (median and range)	Change with HeNe laser (median and range)	*p*-value
Plaque index	−0.5 (0.1 to −1.7)	−0.9 (−0.2 to −1.7)	0.022
Gingival index	−0.6 (−1.0 to −1.7)	−1.8 (−0.3–2.5)	<0.001
Pocket depth, mm	−0.1 (0.2–0.4)	−0.9 (−0.2 to −1.6)	<0.001
GCF volume, µl	−0.06 (0.21 to −0.43)	−0.25 (−0.01 to −0.43)	0.014

$n=20$ patients
Significance of differences calculated with Wilcoxon signed rank test.

Results

Baseline and follow up values for plaque and gingival index are shown in Figs. 1 and 2. Both plaque and gingival index were significantly more reduced on the side treated with HeNe laser ($p=0.022$ and $p<0.001$, respectively) (Table 1). The median baseline probing depth was 4.6 mm on the HeNe laser side and 4.3 mm on the diode laser side. After treatment, the probing depth was 3.5 mm on the HeNe laser side and 4.2 mm on the diode side

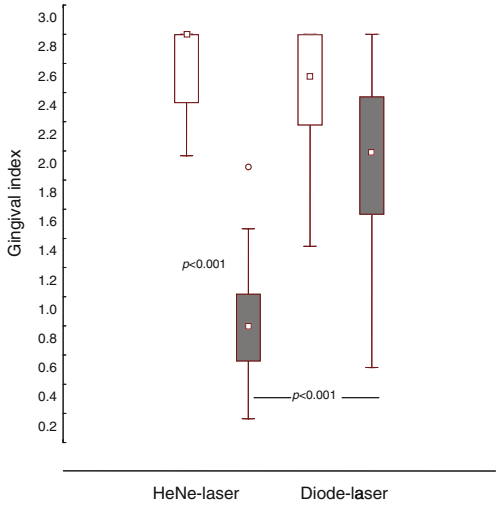

Fig. 2 Gingival index before and after laser treatment. *Filled boxes* indicate the results after treatment. The *box plots* show median, 75 and 90% range and outliers. Indicated *p*-values calculated with Wilcoxon signed rank test

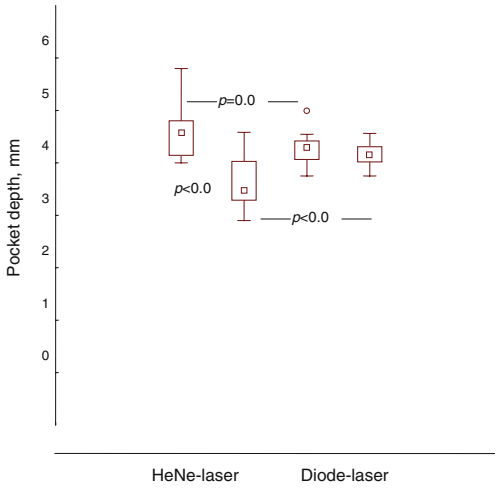

Fig. 3 Pocket depth (mm) before and after laser treatment. *Filled boxes* indicate the results after treatment. The *box plots* show median, 75 and 90% range and outliers. Indicated *p*-values calculated with Wilcoxon signed rank test

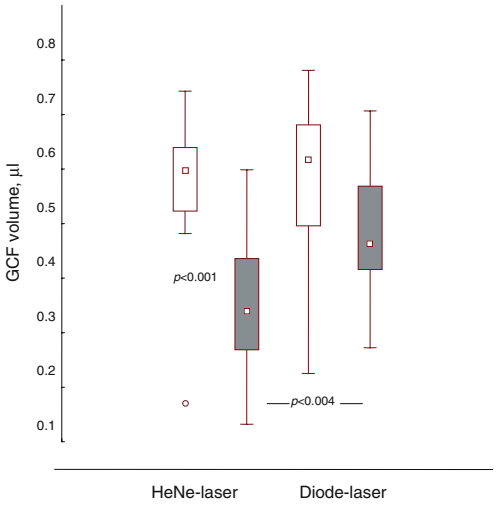

Fig. 4 Gingival crevicular fluid volume (GCF) (µL) before and after laser treatment. *Filled boxes* indicate the results after treatment. The *box plots* show median, 75 and 90 % range, and outliers. Indicated *p*-values calculated with Wilcoxon signed rank test

(Fig. 3). The probing depth reduction was significantly larger on the HeNe laser side (Table 1). The gingival crevicular fluid volume decreased more on the HeNe laser side (Fig. 4 and Table 1).

The laboratory analyses showed no significant effect of the laser irradiation on the content of IL-8 and MMP-8 in GCF (Table 2). The reduction of MMP-8 was more pronounced on the side that had been treated with HeNe laser, but the difference between the two lasers was not significant (p=0.066). With regard to the subgingival microbiota, no differences were detected between the two lasers in the frequencies of positive subjects or of subjects with $>10^6$ of the 12 bacteria analysed.

Discussion

The clinical signs of inflammation, such as gingival index and probing pocket depth, were significantly more reduced on the side given treatment with the HeNe laser compared to the side treated with diode laser. The results in this study are in line with those reported by Kiernicka et al. [32], although 830 nm laser light was used in that study, compared to 632.8–650 nm in the current investigation. The optical parameters are important in laser phototherapy, and this may explain the conflicting results in the studies on gingivectomy by Amorim et al. [24], Damante et al. [33] and Mousques [34].

A number of studies have compared the biological effect of coherent and incoherent light, and all of them indicate that the effect of light from lasers is superior to noncoherent light [35]. (With noncoherent light, we mean light with very low degree of coherency, such as light from LED or filtered halogen lamps.). In a study by Rosner et al. [36], the effect of HeNe laser in the regeneration process of crushed optical nerves was estimated. While HeNe laser postponed the degenerative process, noncoherent infrared light was ineffective or affected the injured nerves adversely. Other studies [37–41] have also compared coherent and incoherent light and have drawn similar conclusions. Karu et al. [42, 43] has studied the importance of different light characteristics in cell stimulation, such as wavelength, coherence, dose and time regimen. In these studies, coherence had no additional effect. However, these were all performed in vitro on cell monolayers. The cells are here "naked", and there is no scattering in the medium and practically no speckle formation, so the in vitro situation is quite different from the clinical environment, as suggested in the experiments quoted above. Thus, coherence mainly seems to be an important parameter in light stimulation in bulk tissue, which is also pointed out by Karu.

Table 2 Median values (inter-quartile range) of interleukin 8 (IL-8) (pg/site) and collagenase-2 (MMP-8) (ng/site) before and after treatment in 20 patients

Parameters	IL-8			MMP-8		
	Diode laser	HeNe laser	*p*-value[a]	Diode laser	HeNe laser	*p*-value[a]
Baseline	74.5 (40.1)	77.8 (36.1)	Ns	8.3 (10.6)	9.0 (11.2)	Ns
Follow up	43.1 (50.1)	52.0 (44.6)	Ns	8.0 (9.4)	5.5 (9.3)	Ns
p-value[b]	Ns	Ns		Ns	Ns	
Change	−36.6 (80.2)	−21.7 (69.9)	Ns	−0.7 (13.7)	−2.8 (6.0)	Ns
Change %	48.4 (85.4)	32.2 (88.2)	Ns	−8.6 (149.9)	−44.9 (101.6)	Ns*

Significance of differences calculated with Wilcoxon signed rank test.
*p=0.066
[a] *p*-value indicates significance of difference between diode laser and HeNe laser.
[b] *p*-value indicates significance of difference between baseline and follow up.

In this study, the irradiation with HeNe laser also reduced the amount of supragingival plaque more than the diode laser, while neither of the lasers had an obvious effect on the subgingival microflora. These findings are in agreement with earlier reports [26, 44].

A new explanation of the action of coherent light in tissue, contributing to the understanding of biological activity caused by low-level laser radiation, has been suggested by Rubinov [45]. It is based on the dipole interaction of gradient laser fields with cells, organelles and membranes. The laser intensity gradients in an object arise due to the interference of the light, scattered by the tissue with the incident light beam (speckle formation). It is shown that gradient laser fields may cause spatial modulation of the concentration of particles and increase their partial temperature. Incoherent light does not cause speckle formation. In the discussion about the mechanisms behind laser phototherapy, usually, absorption of light in photoreceptors, such as porphyrines and cytochrome-*c* oxidase, has been mentioned as the most important factor. However, with the explanation above in mind, an effect on the cell can also be exerted through the gradient forces induced by the coherent light in itself.

A weakness in this study is the difference in wavelength. Although the difference is small, 17 nm, it may not be negligible. The biological differences for irradiation at 633 and 650 nm are not well documented. Nascimento et al. [46] has compared the differences between 670 and 685 nm diode laser irradiation on wound healing in rats, using the same dose but three different powers for each wavelength. All six groups healed better than the control group, and although microscopically all were slightly different, the differences in result between the two wavelengths were not great. The exact influence of the wavelength cannot be extracted from that study, but it still underlines the delicate response from the cells. As for the clinical considerations of the present study, it is documented that all clinical parameters were improved on the HeNe side, while the results of the diode laser were less pronounced.

Further studies should be performed using more exact laser parameters and at different doses. It seems that the HeNe laser dosage lies within the therapeutic window. The less pronounced results of the diode laser might be explained by the assumption that light of low coherency requires higher doses than light from highly coherent sources. Increasing the dose of the diode laser may, in that case, provide results similar to the HeNe laser.

Conclusion

The results from the present study suggest that there is a difference in the biological effect between lasers of long and short coherence length and that the lasers of longer lengths of coherence have a stronger stimulating effect.

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III

A Short-Term Evaluation of Nd:YAG Laser as an Adjunct to Scaling and Root Planing in the Treatment of Periodontal Inflammation

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Background: This split-mouth, single-masked, randomized, controlled clinical trial compares the short-term outcomes of a combined treatment with scaling and root planing (SRP) and neodymium-doped:yttrium, aluminum, and garnet (Nd:YAG)–laser irradiation with treatment with SRP alone.

Methods: Thirty patients were recruited. The mandibular left or right side was randomly assigned as the test side (SRP with laser treatment) or control side (SRP alone). The water-cooled Nd:YAG laser was used at 4 W, 80 mJ/pulse, 50 Hz, and with a pulse width of 350 μs. At baseline, gingival crevicular fluid (GCF) samples were taken from the test and control sides, and levels of matrix metalloproteinase (MMP)-8 and interleukin (IL)-1β, -4, -6, and -8 were measured using standard techniques. The plaque index (PI), gingival index (GI), and probing depth (PD) were measured by calibrated examiners.

Results: At the 1-week follow-up, PD (P <0.001), PI (P <0.05), and GCF volume (P <0.001) showed significant improvement on test sides compared to control sides. At the 3-month follow-up, PD (P <0.01), PI (P <0.01), GI (P <0.01), and GCF volume (P <0.05) also showed significant improvement on test sides compared to control sides. At the 1-week follow up, IL-1β and MMP-8 levels were significantly reduced on test sides compared to control sides. The 3-month follow-up confirmed that the improvements on test sites had been sustained compared to the treatment outcomes of control sites.

Conclusion: In the short-term, SRP in combination with a single application of a water-cooled Nd:YAG laser significantly improves clinical signs associated with periodontal inflammation compared to treatment with SRP alone. *J Periodontol 2010;81:1161-1166.*

KEY WORDS

Cytokines; dental scaling; inflammation; Nd:YAG; root planing.

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The neodymium-doped:yttrium, aluminum, and garnet (Nd:YAG) laser has been used in dentistry, primarily in minor surgery and endodontics, for nearly 2 decades.[1,2] Several potential roles for lasers in periodontal treatment were proposed, such as the removal of calculus, the epithelial lining of periodontal pockets, and granulomatous tissue.[3-7] However, the reported outcomes of such interventions are contradictory.[8] Consequently, laser periodontal therapy has yet to achieve the status of a routine treatment modality. It was reported that Nd:YAG and erbium-doped:yttrium, aluminum, and garnet lasers may be comparable to scaling and root planing (SRP) with respect to reducing periodontal inflammatory conditions.[9] However, other studies[10-12] reported limited evidence to support the efficacy of laser treatment as an adjunct to non-surgical periodontal treatment in adults with periodontal inflammation. This lack of consensus among studies could partly be attributed to a lack of conformity in study methods including laser settings (water cooling, power output, pulse-repetition rate, and fiber diameter) and study design.

Theoretically, the Nd:YAG laser has a potential application in periodontal therapy because the wavelength is not readily absorbed by hard tissues such as cementum or dentin. Within the dose

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ranges recommended for clinical application, the Nd:YAG laser (even without water cooling) only affects the soft tissues such as the pocket epithelial lining.[3] Israel et al.[13] showed that the use of high energy powers, such as 9 W, can have negative effects on root surfaces. However, Spencer et al.[14] reported that the use of the Nd:YAG laser at 4 W is safe and does not have damaging effects on root surfaces.

An unresolved issue is that the Nd:YAG laser may cause overheating of the irradiated tissues.[11] Earlier studies[15-17] used a laser instrument (without water cooling) with a probe diameter of 300 μm for periodontal therapy, which may expose the oral tissues to thermal damage. However, using a laser instrument with a probe diameter of 600 μm (with water cooling) may relatively reduce the risk of thermal damage to periodontal tissues and root surfaces. Another advantage of a larger-diameter instrument tip (with water cooling) is that the energy density at the laser tip is reduced, and the water irrigation reduces the clogging of the probe with debris, thereby preventing a buildup of areas of excessive heat.

The aim of the present short-term study is to test the hypothesis that a water-cooled Nd:YAG laser (wavelength: 1,064 nm) as an adjunct to SRP in the treatment of periodontal inflammation can improve periodontal healing.

MATERIALS AND METHODS

Patient-Selection Criteria

In April, 2005, in Enköping, Sweden, 30 adults (13 males and 17 females, age range 26 to 70 years; mean age: 50 years) were questioned about their systemic health status, use of medications, and tobacco habits.

Inclusion and Exclusion Criteria

To be included in the study, the participants had to have ≥6 periodontal pockets of 4 to 8 mm (periodontal inflammation) on each side of the mandible. Patients were excluded from the study if they had a history of systemic disease requiring medications, received antibiotics within the 12-week period preceding the study, or exhibited class II or III tooth mobility. Based on a previous study[18] involving a therapeutic laser, 25 patients were considered the minimum number of participants.

Ethical Considerations

The protocol was explained to the patients, and volunteering individuals were requested to sign a consent form. The study was approved by the Regional Ethics Review Board, Stockholm, Sweden.

Periodontal Examination

At baseline, two trained and calibrated examiners (PP and FJ), who were masked to the test and control groups, measured the clinical periodontal parameters (i.e., probing depth [PD],§ gingival index [GI],[19] and plaque index [PI][20]) on all mandibular teeth excluding third molars. These measurements were recorded on four sites per tooth (mesial, distal, buccal, and lingual). Oral hygiene instructions were given to all participants on enrollment and at the two treatment sessions.

Treatment Protocols

Patients underwent two different treatment modalities. The teeth on test sides of the mandible received SRP and laser treatment, whereas control sides were treated with SRP alone. The assignment of the left or right side for the respective treatments was randomly determined by a coin toss prior to initiating therapy. Prior to treatment, baseline gingival crevicular fluid (GCF) samples were procured for teeth #19, #20, #29, and #30.

Under local anesthesia, all mandibular teeth, excluding third molars, were scaled and root planed using hand‖ and ultrasonic¶ instruments. All treatments were carried out by one operator (TQ), whereas the baseline and follow-up examinations were performed by two observers (PP and FJ). Follow-up examinations were performed 1 week and 3 months after the final treatment by the same observers.

At the follow-up appointments, patients were questioned concerning the occurrence or lack of any adverse events related to treatment.

Laser Parameters

The laser treatment was accompanied by air and water cooling. The irradiation parameters were determined through the fiber diameter, treatment time, power of the laser at the tip of the fiber, and the surface area of the irradiation site. The laser treatment was performed by inserting the fiber into the periodontal pocket almost parallel to the tooth and moving from mesial to distal directions continuously. The distal end of the laser probe was used to transfer the radiation because this surface was presumed to have sufficient energy to reduce inflammation. The laser equipment used in this study was an Nd:YAG# laser that emitted pulsed light at 1,064 nm. To avoid the thermal effect and maintain the optimal therapy effect, the instrument was set at level five at the following parameters: average output: 4 W; energy per pulse: 80 mJ; pulse width: 350 μs, pulse-repetition rate: 50 Hz; pulse peak power: 240 W; average power density at the fiber end: 1,430 W/cm^2; and peak-power density: 85,800 W/cm^2. The laser energy per treated tooth was 240 to 480 J. The fiber diameter

§ PerioWise, Premier Products, Plymouth Meeting, PA.
‖ American Eagle Curette, Missoula, MT.
¶ Sonosoft Lux, KaVo Dental, Biberach, Germany.
# Genius Dental, Tureby, Denmark.

was 600 μm (0.002826 cm^2). Water cooling and air cooling were always used during irradiation. The time spent on each tooth varied between 60 to 120 seconds, depending on accessibility. The fiber was held in a constant motion in contact with the pocket epithelial lining almost parallel to the long axis of the root. The power density and peak-power density were calculated by a hypothetical 100% emission through the small fiber tip. However, the energy was not emitted solely from the tip of the fiber; there was also considerable lateral emission. Because of the high uncertainty about the total area of irradiated tissue, the energy density (joules per square centimeter) was not calculated.

GCF Collection

Baseline GCF samples were collected from teeth #19, #20, #29, and #30. Prefabricated paper strips** were inserted into the pockets until resistance was felt and were removed after 30 seconds. If the GCF sample was contaminated with blood, it was discarded, and fresh samples from the same site were collected after an interval of 10 minutes. In total, ~10 blood-contaminated samples were discarded.

The collected volume was measured with a calibrated electronic gingival fluid measuring device.†† The two samples from the same side were pooled and eluted in 1 ml phosphate buffered saline for 60 minutes prior to freezing at –20°C.

Analysis of GCF Samples

GCF samples from test and control sites were analyzed for the concentrations of interleukin (IL)-1β, -4, -6, and -8 and matrix metalloproteinase (MMP)-8. These cytokines were analyzed using standard techniques.‡‡§§ The results were calculated using a software program,‖‖ and the cytokine levels were determined as the total amount per site in picograms in the fluid. The collagenases were analyzed similarly using a kit.¶¶

Statistical Analyses

All statistical analyses were performed using a software program.## Changes in the clinical parameters from baseline to follow-up and between treatment modalities were assessed for statistical significance using a paired *t* test. The corresponding differences in laboratory data were analyzed using the Wilcoxon signed-rank test. Significance was set at $P < 0.05$.

RESULTS

All 30 participants attended the baseline examination and the follow-up appointments. The test and control sides included 201 teeth (487 sites) and 204 teeth (494 sites), respectively. Five patients were smokers, and one patient used smokeless tobacco.

Clinical Outcomes

One week post-treatment, the PI ($P < 0.05$), PD ($P = 0.001$), and GCF volumes ($P < 0.001$) significantly decreased at test sides compared to at control sides. The GI also decreased on test sides, but the difference did not reach significance (Table 1).

The 3-month follow-up confirmed that the improvements were sustained. The treatment outcomes for test sites had significantly improved compared to the treatment outcomes for control sites (PD [$P < 0.01$], GI [$P < 0.01$], PI [$P < 0.01$], and GCF volume [$P < 0.05$]) (Table 1). During the 3-month follow-up, the mean PD decreased by 0.6 mm on test sides compared to control sides.

None of the participants reported any adverse side effects that could be related to the laser irradiation.

Laboratory Variables

One week post-treatment, the IL-1β ($P < 0.05$) and MMP-8 ($P < 0.05$) levels were significantly reduced on test sides compared to control sides (Table 2). With respect to the other cytokines, no significant differences were disclosed between the two treatment modalities (Table 2).

DISCUSSION

In the present study, sites irradiated with the Nd:YAG laser as an adjunct to SRP exhibited enhanced periodontal healing compared to sites treated by SRP alone. Improvement in all the registered periodontal variables, including GCF volume, was greater for the irradiated sites than for control sites. The mean PD after the 3-month follow-up had decreased by 0.6 mm on test sides compared to control sides. The gingival inflammation, measured as GI, decreased on both sides, but the decrease was significantly larger on the laser side after 3 months. The combination of reduced GI and reduced PD was an indication of decreased periodontal inflammation.

In contrast, a study by Sjöström and Friskopp[21] that used a similar Nd:YAG laser (with water cooling) immediately after SRP disclosed no additional benefit for laser irradiation at the control side at 4 months. The reason for the discrepancy between the two studies is unclear; however, it might be attributable to differences in the laser settings: in the earlier study,[21] the laser was set to 7 W in accordance with the manufacturers' recommendations, whereas in the present study, the setting was lower (at 4 W).

** PerioPaper, Oraflow, Plainview, NY.
†† Periotron, Oraflow.
‡‡ Luminex, Austin, TX.
§§ Linco Research, St. Charles, MO.
‖‖ Bio-Rad Laboratories, Hercules, CA.
¶¶ Systems Europe, Abingdon, U.K.
STATISTICA v. 6.0, StatSoft, Tulsa, OK.

Table 1.

Periodontal Inflammatory Parameters (mean [SD]) in 30 Patients

	SRP					SRP Plus Nd:YAG-Laser Irradiation				
Variable	Baseline	1 Week	Change 1*	3 Months	Change 2†	Baseline	1 Week	Change 1*	3 Months	Change 2†
PD (mm)	4.41 (0.27)	3.88 (0.42)	–0.53 (0.30)	3.57 (0.48)	–0.84 (0.39)	4.59 (0.44) $P = 0.012$‡	3.61 (0.48) $P = 0.004$‡	–0.98 (0.44) $P = 0.001$‡	3.12 (0.60) $P = 0.001$‡	–1.47 (0.46) $P < 0.01$
PI	1.96 (0.68)	1.26 (0.76)	–0.70 (0.59)	1.48 (0.57)	–0.48 (0.69)	2.02 (0.65)	1.05 (0.70) $P = 0.05$‡	–0.97 (0.67) $P < 0.05$‡	1.11 (0.69) $P < 0.01$‡	–0.91 (0.81) $P < 0.01$‡
GI	1.97 (0.53)	1.46 (0.54)	–0.51 (0.58)	1.53 (0.54)	–0.43 (0.55)	2.11 (0.65)	1.40 (0.58)	–0.72 (0.50) $P = 0.07$‡	1.10 (0.60) $P < 0.001$‡	–1.02 (0.76) $P < 0.01$‡
GCF volume (μl)	1.40 (0.31)	1.53 (0.34)	0.13 (0.36)	1.26 (0.41)	–0.14 (0.45)	1.44 (0.38)	1.12 (0.40) $P < 0.001$‡	–0.32 (0.47) $P < 0.001$‡	1.04 (0.41) $P < 0.05$‡	–0.40 (0.47) $P < 0.05$‡

* Differences in variables from baseline to the 1-week follow-up.
† Differences in variables from baseline to the 3-month follow-up.
‡ Significant differences between the two treatment groups (paired *t* test).

Table 2.

Levels of Cytokines (median ranges) in Pooled GCF Samples (N = 30)

	SRP					SRP Plus Nd:YAG-Laser Irradiation				
Cytokines (pg)	Baseline	1 Week	Change 1*	3 Months	Change 2†	Baseline	1 Week	Change 1*	3 Months	Change 2†
IL-1β	0.32 (0.89)	0.42 (0.84)	0.02 (0.48)	0.18 (0.33)	–0.20 (0.78)	0.46 (1.35)	0.24 (0.71)	–0.26 (1.66) $P < 0.05$‡	0.12 (0.71)	–0.08 (0.77)
IL-4	0.66 (2.04)	0.21 (1.26)	–0.30 (1.07)	0.23 (2.01)	–0.09 (0.689)	0.31 (2.81)	0.54 (2.94)	–0.06 (0.33)	0.03 (2.17)	–0.17 (0.31)
IL-6	0.08 (0.49)	0.0 (0.31)	0.0 (0.32)	0.0 (0.08)	0.0 (0.40)	0.10 (0.56)	0.0 (0.70)	0.0 (0.43)	0.0 (0.20)	0.0 (0.38)
IL-8	84.6 (80.8)	89.0 (86.9)	–5.4 (41.6)	59.0 (85.2)	–14.7 (76.6)	100.0 (95.8)	44.6 (74.9)	–33.0 (100.9)	45.6 (81.4)	–28.7 (53.9)
MMP-8	7.00 (29.5)	9.60 (33.2)	1.56 (8.4)	5.70 (14.0)	–1.89 (31.4)	12.9 (37.4) $P < 0.05$‡	6.91 (29.4)	–5.6 (23.9) $P < 0.05$‡	2.70 (14.8)	–4.88 (34.9)

* Change from baseline to the 1-week follow-up.
† Change from baseline to the 3-month follow-up.
‡ Significant differences between the two treatment groups (paired *t* test).

The disruption of collagen fibers in the periodontal ligament is mainly attributed to the two collagenases MMP-1 and MMP-8. MMP-8 is released primarily from polymorphonuclear leukocytes (PMNs) and secreted predominantly into the GCF. The level of MMP-8 in a GCF sample reflects the number of PMN present and is an expression of the severity of inflammation.[22]

IL-1β is a proinflammatory cytokine that is mainly released from monocytes/macrophages, and is present in the gingival tissues and GCF of patients with periodontal inflammation.[23] In the present study, a significantly greater reduction in MMP-8 and IL-1β was associated with the laser irradiation. Thus, the laboratory analyses confirm the clinical signs of improved healing at these sites. A study by Liu et al.[24]

compared the effects of SRP and SRP plus Nd:YAG laser on the laboratory markers of periodontal inflammation. The 6- to 12-week follow-up results showed a significant reduction in IL-1β levels after treatment with SRP plus the Nd:YAG laser compared to treatment solely with SRP.[24] Similar results were reported by other studies.[7,25,26]

Studies[13,14,27] have compared the effects of ultrasonic treatment, carbon-dioxide–laser treatment, and Nd:YAG-laser treatment. Compared to the baseline values, treatment with the Nd:YAG laser (without water cooling) and ultrasonic scaling resulted in significant improvements in clinical parameters.[13,14,27]

In vivo, effects on the root surface and the pulp are not well documented.[11,28] The effect of laser irradiation on the surrounding tissues is influenced by parameters such as power, pulse, fiber size, fiber angulations, and cooling/no cooling. A study by White et al.[29] suggested that powers from 0.3 to 3.0 W should not cause a damaging rise in intrapulpal temperatures. Likewise, Spencer et al.[14] reported that the use of the Nd:YAG laser at 4 W is safe and does not have damaging effects on root surfaces.

The laser fiber used in the present study was 600 μm in diameter and was operated with a water-cooling system. Compared to a 600-μm tip, the power density of the conventional 300-μm tip is four times higher, which causes greater carbonization and tissue adherence and results in less control over the energy output at the tip. The 600-μm tip reduces the power density, and so does the water spray.[3,6] In the present study, to overcome the loss of power at the fiber tip, the following settings were selected: 4 W, 80 mJ/pulse, 50 Hz, and a pulse width of 350 μs. A further advantage of the 600-μm tip is the reduced risk of fiber fracture. Results by Israel et al.[13] showed that the use of high-energy powers, such as 9 W, can have negative effects on root surfaces. However, if laser treatment is provided with water cooling at 4 W, there is no damage to root surfaces.[14]

It is difficult to provide an absolute explanation for the improvement of periodontal status on the test sites compared to control sites; however, the partial removal of the pocket epithelial lining may be an important contributing factor. Simultaneously, the reduction in PI[30] and PD in the test sites may be explained by the decrease in periodontal inflammation in these sites. This might have reduced the patients' discomfort in these sites and allowed them to brush and maintain their oral hygiene in these areas.

CONCLUSION

The 3-month post-treatment results of this study indicate that treatment with SRP in combination with the Nd:YAG laser is more effective in reducing periodontal inflammation compared to treatment solely by SRP.

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IV

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ORIGINAL ARTICLE

Long-term effects of a single application of a water-cooled pulsed Nd:YAG laser in supplement to scaling and root planing in patients with periodontal inflammation

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Abstract The aim of this work was to investigate the long-term effects of a single application of a water-cooled pulsed neodymium yttrium aluminium garnet (Nd:YAG) laser, in combination with scaling and root planing (SRP) for the treatment of periodontal inflammation. Twenty-two patients were included in this split-mouth single blind randomized controlled clinical trial. The parameters of the air and water-cooled Nd:YAG laser were: 4 W, 80 mJ/pulse, 50 Hz and a pulse width of 350 μs. The "test side" was treated with a single application of Nd:YAG laser and SRP; while the "control side " was treated with SRP alone. At baseline, and after a median follow-up time of 20 months (range 12–39), periodontal inflammatory parameters (plaque index [PI], gingival index [GI], probing pocket depth [PPD]), and marginal bone loss (on digital bite-wing radiographs) were measured. Gingival crevicular fluid (GCF) was collected from the teeth 35, 36, 45, and 46 at baseline and at follow-up. Pl ($p<0.01$), GI ($p<0.01$), and PPD ($p<0.001$) were significantly lower on the test side compared to the control side at follow-up. Radiological results showed significantly less bone loss on the test side compared to the control side ($p<0.05$). GCF volume was lower on the test side compared to the control side ($p<0.01$). In conclusion, a single application of Nd:YAG laser in combination with SRP had a positive long-term effect on periodontal health compared to treatment by SRP alone.

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Introduction

Lasers are used for periodontal treatments including removal of calculus, epithelial lining of periodontal pockets, and granulomatous tissue [1–5]. The neodymium-yttrium-aluminium-garnet (Nd:YAG) laser, approved for periodontal treatment by the US Food and Drug Administration, has been in use for periodontal curettage for nearly three decades [6–8].

Theoretically, the Nd:YAG laser has a potential application in periodontal therapy because the wavelength is not readily absorbed by hard tissues such as cementum or dentin. Within the dose ranges recommended for clinical application, the Nd:YAG laser (even without water cooling) affects only the soft tissues such as the pocket epithelial lining [3]. The Chanthaboury and Irinakis study [8] has reported that the Nd:YAG is comparable to scaling and root planing (SRP) in reducing periodontal inflammation. However, there is limited evidence to support the efficacy of laser treatment as an adjunct to non-surgical periodontal treatment in adults with periodontal inflammation [9–11]. A debatable issue is that the Nd:YAG laser may cause overheating of the irradiated tissues and hence expose the soft and hard oral tissues to damage [12]. It should be noted that most previous studies used laser instruments (without water cooling) with an optical fiber of 300-μm diameter [13, 14]. However, the risk of thermal damage to periodontal tissue and the root surface may be evaded by using water-cooled laser instruments with a probe diameter of 600 μm. A larger diameter of the laser tip helps reduce the energy density at the laser tip. Water-irrigation also

reduces clogging of the probe with debris, thereby preventing a build-up of areas of excessive heat.

In this context, the aim of the present study was to assess the long-term efficacy of a water-cooled pulsed Nd:YAG laser (1,064-nm) in supplement to SRP in the treatment of periodontal inflammation.

Materials and methods

The trial was approved by the regional ethics review board in Stockholm, Sweden. The study participants (aged between 26 and 70 years) were recruited for a study of the short-term effects of a combined treatment with scaling and root planning and irradiation with Nd:YAG laser [15]. Consenting individuals underwent a preliminary clinical dental examination and their mandibular probing pocket depths were measured by the main author (TQ). In order to be included, the participants had to have at least six periodontal pockets of 4–8 mm (periodontal inflammation) on each side of the mandible. Subjects were asked about their systemic health, medications, as well as tobacco habits. The exclusion criteria were based on the following: intake of medications for systemic illnesses, use of antibiotics over the previous 3 months, tooth mobility (class II or III), mandibular third molars, and/or patients who had previously undergone laser treatment for periodontal inflammation.

In an attempt to investigate if our previous findings [15] were valid over a longer time, we invited these patients (n= 30) for a follow-up analysis. Twenty-two individuals volunteered to participate in the present study and the duration of follow-up ranged from 12–39 months.

Laser parameters and irradiation

The parameters of the air- and water-cooled pulsed Nd:YAG laser were: 4 W, 80 mJ/pulse, 50 Hz, and a pulse width of 350 μs.

Water and air settings were "9" on the machine. Angulation of the tip was between 20 and 30°. The fiber tip was cleaned after each pocket debridement. Power was automatically controlled by the device. The time spent on each tooth varied between 60 and 120 s, depending on accessibility. The fiber was held in constant motion in contact with the pocket epithelial lining. The power density and peak power density reported above are calculated by a hypothetical 100% emission through the small fiber tip. However, the energy is not emitted solely from the tip of the fiber; there is also considerable lateral emission. Thus, due to the high uncertainty about the actual light-emitting surface and the total area of tissue irradiated, the energy density (J/cm^2) was not calculated.

Clinical periodontal investigations

The patients underwent two different treatment modalities. The teeth on the test side of the mandible received SRP and laser treatment; whereas the control side was treated with SRP alone. Assignment of left or right side for the respective treatments was randomly determined (by tossing a coin) before any treatment. Under local anesthesia, the mandibular teeth from 35, 36, 45, and 46 underwent SRP using hand instruments (American Eagle Curette, Missoula, USA) and ultrasonic scalers (Sonosoft Lux, Kavo Dental, Germany).

SRP and laser treatments were performed by one operator (TQ), while the baseline and follow-up periodontal examinations (plaque index [PI] [16], gingival index [GI] [17], and probing pocket depth [PPD] (Perio Wise, Premier, Canada), were conducted by two calibrated examiners (FJ and PP) who were blinded to the test and control groups.

Measurement of gingival crevicular fluid (GCF) volume and immunological investigations

Trained investigators (FJ and PP) collected the baseline and post-operative GCF samples from the teeth 36, 35, 46, and 45. Supragingival plaque was removed from the sites of GCF collection (mesial pocket of the second premolar and the first molar on the test and control sites) with cotton rolls. The GCF was collected with prefabricated paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA), which were inserted into the pockets until resistance was felt and kept in place for 30 s. Blood-contaminated samples were discarded. The collected volume was measured with a calibrated Periotron 8000 (Oraflow Inc. Plainview, NY, USA).

Radiological investigations

Digital bite-wing radiographs (Siemens, Bensheim/Germany) were taken with the vertical long axis of the hemi-mandible using a software program (Schick, Technologies, Inc., NY, USA). All radiographs were taken by the main author (TQ). Baseline and post-operative mandibular alveolar bone loss were gauged (in millimeters) from the mesial surface of second molars to the distal surface of canine teeth by a trained investigator (FJ and AG). Alveolar bone loss was measured from the cementoenaemel junction (CEJ) to the most apical portion of the alveolar bone. Teeth with indistinct or carious lesions at the CEJ were excluded.

Statistical analyses

Statistical analyses were performed using a software program (Statistica v. 6.0, Statsoft, Tulsa, OK, USA). The paired t test was performed to assess the changes in the clinical parameters from baseline to follow-up, and between

the treatment modalities. *p*-values less than 0.05 were considered as statistically significant. Normality was tested with Kolmogorov–Smirnov test.

Results

In total, 22 patients (nine males and 13 females) with periodontal inflammation with a mean age 50 years were included in the study. Four patients were smokers and one subject used smokeless tobacco. The median follow-up time was 20 months (range 12–39 months).

Clinical and radiological results

At the follow-up examination, PI ($p<0.01$), GI ($p<0.01$), and PPD ($p<0.001$) were significantly lower on the test side compared to the control side. Radiological results showed a significant increase in marginal bone height on the test side compared to the control side ($p<0.05$). These results are summarized in Table 1.

Gingival crevicular fluid volume

GCF volume was significantly lower on the test side (mean change: –0.57 μl, range: –0.4 μl to 1.68 μl) compared to the control side (mean change: 0.15 μl, range: –0.12–1.11 μl) ($p<0.01$). These results are summarized in Table 1.

Discussion

In the current study, sites irradiated with a single application of Nd:YAG laser as an adjunct to SRP showed a reduction in periodontal inflammation and bone loss compared to the control side. The clinical reduction of inflammation measured as gingival index was corroborated by the simultaneous reduction of the GCF volume on the test side compared to the control side [18]. Our present study showed a minor bone loss on the SRP alone side while the side treated with laser and SRP showed some bone gain. This is in line with results from a recent experimental study in rats demonstrating an increase in marginal bone height following laser therapy [19].

Besides reducing the periodontal inflammatory conditions, Nd:YAG laser treatment also supports new connective tissue formation. The Yukna study [20] investigated the effect of Nd:YAG laser therapy in patients with periodontal inflammation. The results showed a significant reduction in PPD with increased clinical attachment levels [20]. An interesting finding of this study was that Nd:YAG laser therapy showed new cementum and connective-tissue

Table 1 Summary of clinical changes in the control and test sites. *p* values were calculated using paired *t* test

	Control-site (SRP alone)			Test-site (SRP with Nd:YAG laser)		
Periodontal Variables	**Base-line (mean ± SD)**	**20-months follow-up (mean ± SD)**	**Change (mean ± SD)**	**Base-line (mean ± SD)**	**20-months follow-up (mean ± SD)**	**Change (mean ± SD)**
Probing pocket depth	4.41 ± 0.31	3.86 ± 0.76	-0.55 ± 0.60 †	4.58 ± 0.47	2.97 ± 0.60	-1.61 ± 0.32 †
Plaque index	1.93 ± 0.69	1.86 ± 0.66	-0.07 ± 0.96	2 ± 0.71	1.35 ± 0.56	-0.64 ± 0.85 *
Gingival index	1.97 ± 0.54	1.80 ± 0.56	-0.16 ± 0.72	2.18 ± 0.62	1.03 ± 0.52	-1.15 ± 0.59 *
Marginal bone loss	2.04 ± 0.49	2.16 ± 0.53	+0.11 ± 0.27	2.12 ± 0.44	2.04 ± 0.50	-0.07 ± 0.41 #
GCF volume	1.41 ± 0.34	1.53 ± 0.42	0.15 ± 0.42	1.45 ± 0.42	0.88 ± 0.51	-0.57 ± 0.57 *

SRP Scaling and root planing, *SD* Standard deviation, *Nd:YAG* Neodymium yttrium aluminium garnet laser (water-cooled pulsed)
† $p<0.001$ * $p<0.01$ # $p<0.05$

formation [21]. It has been shown that the Nd:YAG laser when used at low energy does not cause damage to the cementum and dental pulp. The Radvar study [22] also showed that the Nd:YAG laser does not have a negative influence on cementum; thereby suggesting the formation of new connective tissues around the periodontium.

The present study has obvious weaknesses such as the small number of participants, the relatively long unsupervised and varying observation time, and a lack of positioning devices to standardize the radiographs. Since the patients know which side of the lower jaw that was irradiated with the laser, it is possible that they brushed this side more carefully, but considering the long follow-up time, it is not probable that this had a measurable effect.

A difference in bone level of 0.18 mm is not clinically relevant but it is statistically significant and shows that one treatment with a Nd:YAG laser can have a long-term effect on the alveolar bone.

In conclusion, a single application of a water-cooled pulsed Nd:YAG laser in combination with SRP significantly reduced the severity of periodontal inflammation compared to treatment by SRP alone. However, further human and experimental studies are required to assess the influence of combining Nd:YAG laser with SRP for the treatment of periodontal inflammation.

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The Division of Periodontology, Department of Dental Medicine

THE EFFECT OF THERAPEUTIC AND ND:YAG LASER AS AN ADJUNCT TREATMENT MODALITY IN PERIODONTAL THERAPY

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i sal 4U, plan 4, Institutionen för Odontologi, Alfred Nobels Allé 8, Huddinge

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Abstract

Laser irradiation has been proposed as an adjunct to conventional scaling and root planing in the treatment of periodontitis. However, the reported outcomes of studies to date are contradictory and the literature provides limited evidence to support an additional benefit of laser application. The overall aim of the present thesis was to explore the potential of adjunctive application of therapeutic and surgical lasers to improve treatment outcomes, expressed in terms of clinical, radiographic and immunological parameters.

The present thesis is based on a series of four clinical studies of patients with moderately severe periodontitis, treated by scaling and root planing. Two different types of dental laser were investigated. Therapeutic lasers, which are claimed to stimulate cell regeneration and boost the immune system, were investigated in studies I and II: the general effect was investigated in Study I, while Study II compared the difference between gas and diode lasers in the same spectrum, in order to evaluate the importance of the length of coherence in biostimulation. In studies III and IV, the surgical Nd:YAG laser, which is usually applied for sulcular debridement and pocket decontamination, was evaluated in a novel approach. The test procedure comprised one single application of the laser with water coolant after conventional scaling and root planing. In study III, the outcome was evaluated after 3 months and in Study IV the long term outcome was evaluated, at least one year post-treatment.

The split mouth design was used in all four studies. Study I showed a better clinical outcome on the laser treated side and some improvement in immunological parameters. The results of Study II support the hypothesis that a laser with a long length of coherence is superior to one of a shorter length, although both lasers had some positive clinical effect. In Study III a single application of the Nd:YAG laser as an adjunct to scaling and root planing improved the short-term outcome and Study IV confirmed that this improvement was sustained.

In conclusion, the results of these studies confirm the potential role of laser irradiation as a non-invasive adjunctive to scaling and root planing in the treatment of periodontitis.

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